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Spatial variation of trophic position and growth rate of juvenile striped bass (*Morone saxatilis*) in the Delaware River[1]

Sam C. Wainright, Charlotte M. Fuller, Robert H. Michener, and R. Anne Richards

Abstract: The relative recent growth rate (as marginal scale circulus width) of juvenile striped bass (*Morone saxatilis*) was faster in the oligohaline region of the Delaware River (near the turbidity maximum) than further upstream. Stable isotope analyses ($\delta^{13}C$ and $\delta^{15}N$) of the same fish suggested that this difference in growth rate is associated with a change in trophic position. Although salinity was low (0–5 ppt), the $\delta^{13}C$ values of fish from the oligohaline region (–21.3 to –23‰) suggest that a significant part of their diet may be derived from marine-like sources. All $\delta^{15}N$ values for juvenile striped bass were high relative to previously published $\delta^{15}N$ values for freshwater and marine fish and are suggestive of inputs of sewage-derived nitrogen. Fish from the oligohaline region ($\delta^{15}N$ values of 16–20.6‰) may occupy a lower trophic level than fish collected further upstream ($\delta^{15}N$ values of 22.4–26.4‰) based on the difference between the $\delta^{15}N$ of fish and literature values for particulate organic matter collected in the same area and season. To the extent that trophic position reflects habitat quality, habitat quality for juvenile striped bass varies regionally within the Delaware River estuary.

Résumé : Le taux de croissance récent relatif (échelle marginale de largeur des anneaux) de jeunes bars d'Amérique (*Morone saxatilis*) était plus élevé dans la zone oligohaline du fleuve Delaware (au voisinage du maximum de turbidité) que plus loin en amont. L'analyse des isotopes stables ($\delta^{13}C$ et $\delta^{15}N$) dans le même poisson permet de penser que cette différence de taux de croissance est liée à un changement de position trophique. La salinité était faible (0–5 ppt), mais les valeurs de $\delta^{13}C$ pour le poisson de la zone oligohaline (–21,3 à –23‰) laissent supposer qu'une part importante de leur régime alimentaire provient peut-être de sources de nature marine. Toutes les valeurs de $\delta^{15}N$ pour les jeunes bars d'Amérique étaient élevées en comparaison des valeurs publiées précédemment pour les poissons marins et les poissons d'eau douce, et permettent de penser qu'il y a peut-être eu des apports d'azote provenant d'eaux d'égout. Il se peut que les poissons provenant de la zone oligohaline ($\delta^{15}N$ allant de 16 à 20,6‰) occupent un niveau trophique moins élevé que les poissons prélevés plus loin en amont ($\delta^{15}N$ allant de 22,4 à 26,4‰), compte tenu de la différence entre la valeur de $\delta^{15}N$ mesurée dans le poisson et les valeurs données dans la littérature pour la matière organique particulaire prélevée dans la même zone et pendant la même saison. La qualité de l'habitat des jeunes bars d'Amérique varie selon l'endroit dans l'estuaire du fleuve Delaware, dans la mesure où la position trophique reflète cette qualité.
[Traduit par la Rédaction]

Introduction

The striped bass (*Morone saxatilis*) is an anadromous fish that spawns in brackish and freshwater regions of estuaries and spends its first year or more within the estuary (Bigelow and Schroeder 1953). They enter coastal waters as subadults where they form the basis for important recreational fisheries (Richards and Deuel 1987). Year-class strength of striped bass stocks is highly variable (Boreman and Austin 1985), primarily because of strong and variable density-independent mortality during early life history (Ulanowicz and Polgar 1980; Logan 1985; Uphoff 1989). Variability in mortality rates appears to decline significantly after the post-larval stage (Uphoff 1989). In subsequent juvenile stages, predation is probably the primary source of mortality. Size-dependent vulnerability is well documented in fishes (Brooks and Dodson 1965; Gabriel and Pearcy 1981; Peters 1983; Werner and Gilliam 1984) and implies that growth rate in early stages has a critical influence on survival. Growth, in turn, is influenced by a variety of factors, the most fundamental of which is an adequate food supply.

The Delaware River estuary once supported a large spawning

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S.C. Wainright[2] and C.M. Fuller. Institute of Marine and Coastal Sciences, Rutgers University, Cook Campus, New Brunswick, NJ 08903–0231, U.S.A.
R.H. Michener. Boston University, Stable Isotope Laboratory, Department of Biology, 5 Cummington Street, Boston, MA 02215, U.S.A.
R.A. Richards. National Marine Fisheries Service, National Oceanic and Atmospheric Administration, Northeast Fisheries Science Center, 166 Water Street, Woods Hole, MA 02543, U.S.A.

[1] Contribution No. 95–18 of the Institute of Marine and Coastal Sciences, Rutgers University, New Brunswick, N.J.
[2] Author to whom all correspondence should be addressed. e-mail: swainrig@ahab.rutgers.edu

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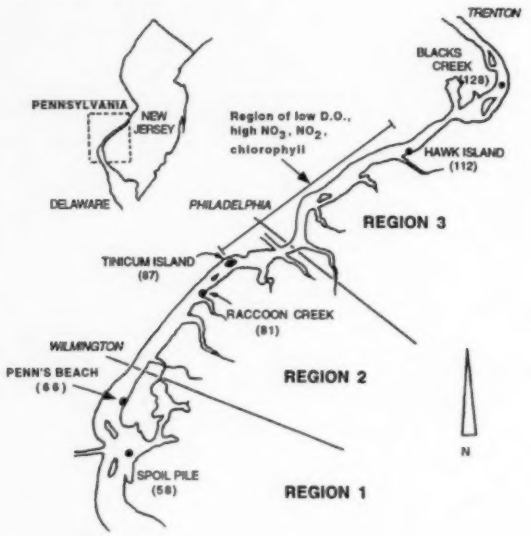
Fig. 1. Map of the Delaware River estuary, showing demarcations of the three regions (modified from Baum 1992), and areas of low dissolved oxygen, high NO_2, NO_3, and chlorophyll *a* (based on Lipschultz et al. 1986).



population of striped bass (Chittenden 1971). During the early 1900s, the stock declined to very low levels, apparently because of hypoxic conditions below the spawning grounds (Chittenden 1971; Albert 1988, see also Lipschultz et al. 1986). Recently, restoration efforts have improved water quality and the striped bass population is showing signs of recovery. A survey of juvenile striped bass conducted since 1980 has shown steadily increasing abundance (Baum 1994), although overall catch rates are still low compared with other estuarine systems (USDI and USDC 1994). Juveniles tend to be most abundant upriver of the saltwater – freshwater interface but are distributed throughout the estuary in habitats that vary from brackish to fresh tidal water. Adults and juveniles also were once abundant in nontidal waters above Trenton, N.J. (Chittenden 1971).

The quality of the various Delaware River habitats occupied by juvenile striped bass is unknown. Are they equally suitable or do some habitats allow more rapid growth and, thus, perhaps a higher probability of survival? This study addressed the question of habitat quality by examining recent growth histories of individual striped bass juveniles from different river regions and correlating them with the trophic position at which the fish were feeding. Trophic position was determined using stable isotope analysis of C and N in fish muscle (Rau et al. 1992; Hobson and Welch 1992; Wainright et al. 1993). Stable isotope ratios of C are an indicator of the source of organic matter (plant material) at the base of the food chain, while nitrogen isotopes provide an estimate of trophic level. Peterson and Fry (1987) give a review of the use of stable isotopes in ecological studies. In this study, it was assumed that food availability per se does not influence stable isotopic composition. It was also assumed that growth rate was an indicator of habitat suitability and that trophic position reflected feeding niche within that habitat.

Methods

Study area

The Delaware River estuary is located between the states of New Jersey, Delaware, and Pennsylvania (Fig. 1). Our study area extended from the head of Delaware Bay upriver to Trenton, N.J. (river miles 58 to 128; 1 mile = 1.6 km). Sampling stations were distributed over three broad regions each approximately 20 km in length. Two stations were sampled in each of the three regions. Salinity ranged from 0 to 5 ppt, with salinities above zero occurring only in region 1 (2 ppt at Penn's Beach, 5 ppt at Spoil Pile (Salem's Cove)). There are inputs of effluent from sewage treatment plants, especially from Trenton downstream to Wilmington, Delaware. Vegetation includes flowering vascular plants upstream and marsh vegetation downstream (mostly *Spartina* sp.; Cifuentes et al. 1991).

Collection

Juvenile striped bass were captured with a 30.5 × 1.8 m beach seine (6.35–mm mesh) by the New Jersey Department of Environmental Protection and Energy during the summer and fall of 1992 at the locations and dates given in Table 1. Fish were placed on ice in the field and stored in a laboratory freezer (–20°C) prior to isotope analysis. After measurement of fork length, samples were collected from each fish for marginal scale measurements and stable isotopic analyses.

Relative growth rate analysis

Relative recent growth rates were determined as marginal increment widths on scales removed from the caudal peduncle (Doyle et al. 1987). The combined width of three circuli was measured beginning one circulus from the margin of the scale. Three scales per individual were measured at 100× on a Zeiss compound microscope with substage lighting, using a calibrated ocular micrometer, and the mean was calculated for each individual. For each of the three regions in the study area, mean relative growth rates were calculated.

Our methods are not dependent on knowing absolute rates of circulus deposition, nor the age at first circulus formation, but on the assumption that circulus deposition is constant, and that intercirculus spacing is proportional to growth rate. These assumptions have been validated for other species in at least three families of fish (Sire 1986; Barber and Walker 1988; Szedlmayer et al. 1991).

Stable isotope analysis

Fish were thawed and a piece of white muscle (ca. 20 mg dry weight) was removed from just below the dorsal fin and above the lateral line. Individual fish of similar fork length from the same collection site were grouped into composite samples before stable isotope analysis (Table 1). The muscle tissue was dried at 30–40°C in a drying oven and ground to a fine powder in a Wig-L-Bug amalgamating machine (Crescent Dental Corp.). The powder was acidified with 1 N HCl to remove inorganic carbon, and re-dried before subjecting it to stable isotope analysis.

Carbon and N stable isotope ratios were determined on separate subsamples of the composite samples. Sample size for each isotopic determination was approximately 1 mg for $\delta^{13}C$ and 3 mg for $\delta^{15}N$. Stable isotope analyses were performed at the Boston University Stable Isotope Laboratory (Boston, Mass.). Samples were flash combusted in tin boats at 1800°C in a Haraeus elemental analyzer and the resulting CO_2 and N_2 gases were separated from the helium stream and purified cryogenically in a Finnigan cold trapping box before introduction into a Finnigan Delta-S isotope ratio mass spectrometer. Samples were measured against high-purity laboratory gas standards that had been calibrated to international standards (Pee Dee Belemnite (PDB) for $\delta^{13}C$ and atmospheric air for $\delta^{15}N$). Values are expressed in delta (δ) notation: $\delta(^{13}C \text{ or } ^{15}N) = (R_{sample}/R_{standard})1000$, where $R = {}^{13}/{}^{12}C$ or $^{15}N/^{14}N$. Replicate analyses of identical composites usually differed by ≤0.1‰ for both C and N stable isotopes.

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Table 1. Inventory of composite samples of juvenile striped bass from the Delaware River Estuary, analyzed for stable isotopes.

Region	Station (RM)	Collection date	Temperature (°C)	*n*	Fork length (cm)		$\delta^{13}C$	$\delta^{15}N$
					Range	Mean		
3	Black Creek (128)	8–4–92	24	5	3.1–3.6	3.40	–24.8	17.3
3	Black Creek	8–4–92		5	4.2–4.9	4.52	–25.1	18.2
3	Hawk Island (112)	9–1–92	25	5	5.8–6.4	6.12	–25.45	22.75
3	Hawk Island	9–1–92		4	4.8–5.1	4.98	–25.8	23.5
2	Tinicum Island (87)	9–8–92	24	2	4.0–4.6	4.30	–24.6	23.4
2	Tinicum Island	9–21–92	22	3	4.9–5.2	5.00	–26.4	23.7
2	Tinicum Island	9–28–92	19	5	5.3–5.6	5.48	–24.7	25.2
2	Tinicum Island	10–14–92	16	4	3.9–4.6	4.33	–26.2	26.4
2	Raccoon Creek (81)	9–8–92	22	3	6.7–7.0	6.87	–24.8	23.0
2	Raccoon Creek	9–8–92		1	13.3	—	–23.5	22.4
1	Penn's Beach (66)	9–15–92	22	2	7.4–7.6	7.50	–23.0	20.5
1	Penn's Beach	9–15–92		1	8.3	—	–22.7	20.6
1	Penn's Beach	9–15–92		2	9.2–9.8	9.50	–22.7	19.6
1	Penn's Beach	9–15–92		2	14.4–15.4	14.90	–21.9	20.0
1	Spoil Pile (58)	9–15–92	23	3	6.9–7.4	7.13	–21.7	17.85
1	Spoil Pile	9–15–92		2	8.3–8.6	8.45	–21.3	16.0
1	Spoil Pile	9–15–92		2	9.1–9.9	9.50	–19.9	17.6
1	Spoil Pile	9–15–92		3	15.0–15.7	15.40	–21.7	19.7

Note: Composite groupings of fish were made for stable isotope analyses. All individuals in each composite were also sampled for scale growth increments (except the first composite listed, because most of the fish in this composite did not have scales). RM, river miles.

Statistical analyses

Analysis of covariance (ANCOVA) was used to test for regional variation in growth rate and stable isotopic composition, while controlling for the potential effect of fish size. Included in the ANCOVA model were region (the main factor), fish size (the covariate), and an interaction term (region × fish size). For the ANCOVA involving growth rate, the size of individual fish was used; for those involving stable isotopes, the mean fish size for the composite was used. All data met conditions of normality and homogeneity of variance. In ANCOVAs for which the interaction term was significant (growth, $\delta^{15}N$), multiple slopes were allowed. In cases where the interaction term was not significant ($\delta^{13}C$), a common slope was assumed and a two-way ANOVA was conducted.

The sampling design was balanced, with each region represented on each date. However, fish were not collected in every region on every date.

Within region 2, the Tinicum Island station was sampled on four occasions, allowing temporal relationships to be explored with correlation analysis. Additionally, two stations within region 1 (Penn's Beach and Spoil Pile) were sampled on the same date; differences between those stations with regard to fish growth rate and stable isotopic composition were examined with two-tailed *t* tests.

Results

Relative growth rates

Growth rates varied regionally (ANCOVA: $F = 5.23$, df = 5, 43, $p = 0.009$). Fish from region 1 had significantly higher growth rates than fish from region 3 (multiple comparison, $p = 0.012$; Fig. 2A). Growth rates were not significantly different between regions 1 and 2 or between regions 2 and 3. There was no significant relationship between fish size and growth rate (Fig. 3). However, there was a significant fish size × region interaction ($p = 0.019$), driven by two individuals from region 2: one fish was unusually large, and one had an unusually high growth rate (Fig. 3A). When these two outliers were removed from the analysis, the size and size × region interaction terms became insignificant. However, all data were included in the analyses presented in this paper.

Stable isotopic composition

Carbon stable isotopic compositions varied regionally (two-way ANOVA; $F = 28.88$, df = 3, 14, $p = 0.0001$). Fish size and the fish size × region interaction were not significant factors in determining $\delta^{13}C$ (Fig. 3; ANCOVA, $p = 0.158$ and 0.361, df = 5 and 12, respectively). Fish from region 1 showed the most positive $\delta^{13}C$ values (mean = –22‰; Fig. 2B). Isotope values of fish from further upstream (regions 2 and 3) were significantly more negative.

Nitrogen stable isotopes also varied regionally (ANCOVA; $F = 9.59$, df = 5, 12, $p = 0.003$), but with a different pattern than $\delta^{13}C$ values. The $\delta^{15}N$ values were highest in region 2, with lower values both upstream and downstream; however, all $\delta^{15}N$ values were high relative to those previously reported by Fry (1988), Harrigan et al. (1989), Goering et al. (1990), Kline et al. (1990), Sholto-Douglas et al. (1991), Hobson and Welch (1992), Rau et al. (1992), Wainright et al. (1993), and Welch and Parsons (1993) for marine systems and by Estep and Vigg (1985), Hesslein et al. (1991), and Cabana et al. (1994) for freshwater systems (Fig. 2C). In contrast with the $\delta^{13}C$ data, fish $\delta^{15}N$ values were influenced by fish size ($p = 0.035$; Fig. 3), and there was a significant fish size × region interaction ($p = 0.034$). Fish from region 1 were significantly different from those from regions 2 ($p = 0.0004$; multiple comparisons) and 3 ($p = 0.01$), while fish from region 2 were not different from those from region 3 (Fig. 2C).

Within region 1, growth rates were similar at the two stations that were sampled on 15 September 1992 (Fig. 2A; mean circulus width at Spoil Pile: 0.045 ± 0.003 mm (mean ± SD); 0.046 ± 0.003 mm at Penn's Beach). However, both C and N stable isotope values were significantly different. Carbon stable isotope values (Table 1) were higher at Spoil Pile (mean =

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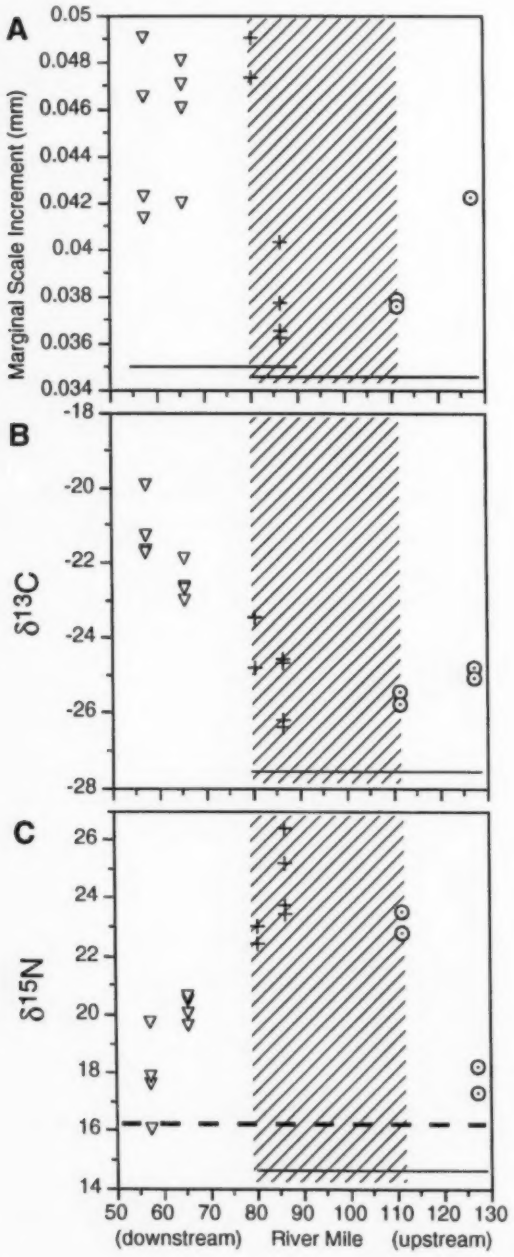
Fig. 2. (A) Relative recent growth rates of composite juvenile striped bass samples from the three sampled regions of the Delaware River, as a function of river mile. Lines beneath the data join regions that do not differ significantly (multiple comparison test; $\alpha = 0.05$); sample sizes were 17, 18, and 14 for regions 1, 2, and 3, respectively. (B) and (C) as (A), but for $\delta^{13}C$ and $\delta^{15}N$, respectively. Symbols: region 1, ▽; region 2, +; region 3, ⊙. The shaded area represents the zone of elevated NO_2, NO_3, and chlorophyll *a*, and depressed dissolved oxygen shown in Fig. 1 (from Lipschultz et al. 1986). In (C), the dotted line represents the upper limit of $\delta^{15}N$ values for muscle tissue previously reported for freshwater and marine fish (see text for references). In (B) the $\delta^{13}C$ for striped bass reported here are within the range of values reported by studies cited above.



–21.15‰) 8 miles downstream from Penn's Beach (mean = –22.58‰; $t = -2.920$, df = 6, $p = 0.027$). The $\delta^{15}N$ values were lower at Spoil Pile (mean = 17.79‰) than at Penn's Beach (mean = 20.18‰; $t = 3.012$, df = 6, $p = 0.024$).

The Tinicum Island station (region 2) was sampled on four different dates (Table 1). Relative growth rates showed no temporal pattern at that station nor did $\delta^{13}C$ values. However, $\delta^{15}N$ values were positively correlated with time ($r^2 = 0.913$, $n = 6$, $p = 0.044$; Fig. 4).

Discussion

Numerous factors in estuaries are likely to influence the growth rates of juvenile fish, including physical factors (Morris et al. 1978; Sharp et al. 1984; Fisher et al. 1988; Dodson et al. 1989; Connaughton et al. 1994; Lankford and Targett 1994) and factors relating to feeding ecology, e.g., trophic level, feeding strategy, or food availability (Goshorn and Epifanio 1991; Pryor and Epifanio 1993; Welch and Epifanio 1995). Our data suggest that some combination of physical and biological factors is responsible for the regional differences in relative growth rates and isotopic compositions that we observed.

The region of maximal growth rate (region 1) corresponds with the turbidity maximum in the Delaware estuary, in the oligohaline zone (Sharp et al. 1984; Fisher et al. 1988). Enhanced growth in this area suggests that some of the elevated particle load associated with the turbidity maximum may be biologically labile (Morris et al. 1978; Dodson et al. 1989). The large variation in growth rate within region 2 suggests that this region too may represent part of a transition zone, possibly resulting from high variability in habitat quality within that region or from the movement of fish between regions 1 and 2 (Fig. 2A). Our marginal circulus method has not yet been calibrated relative to growth rates in juvenile striped bass. However, in *Tilapia*, each circulus corresponds with approximately 4–7 days growth (Doyle et al. 1987), and rings are deposited daily in juvenile *Cynoscion regalis* up to a standard length of 8.2–14.2 cm (Szedlmayer et al. 1991). Therefore, growth rates determined in this study may integrate growth over time periods of days to weeks.

Size data and collection dates presented in Table 1 suggest the possibility of a downstream migration pattern with time. The smallest fish occurred upstream (region 3) early during the study and were not collected in that region later in the season. Fish from regions 1 and 2 were larger and were collected during all but the first month of the study. In contrast, a recent mark–recapture study conducted within region 2 found no evidence of a regular migration pattern (Burton and Weisberg 1994, Fig. 2). However, their study used somewhat larger marked fish (6–14 cm) and a more restricted study area.

Our $\delta^{15}N$ and $\delta^{13}C$ results indicate that striped bass occupy a different trophic position (feeding niche) in different areas within the Delaware River estuary. Even on a small spatial scale within region 1 (ca. 8 river miles) significant isotopic differences were observed. Although striped bass were not

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Fig. 3. Relative recent growth rate (A), $\delta^{13}C$ (B), and $\delta^{15}N$ (C) as functions of fish size for the three sampled regions in the Delaware estuary. Symbols: region 1, ▽; region 2, +; region 3, ⊙. Plotted points in A are individual fish; those in B and C are composite samples.

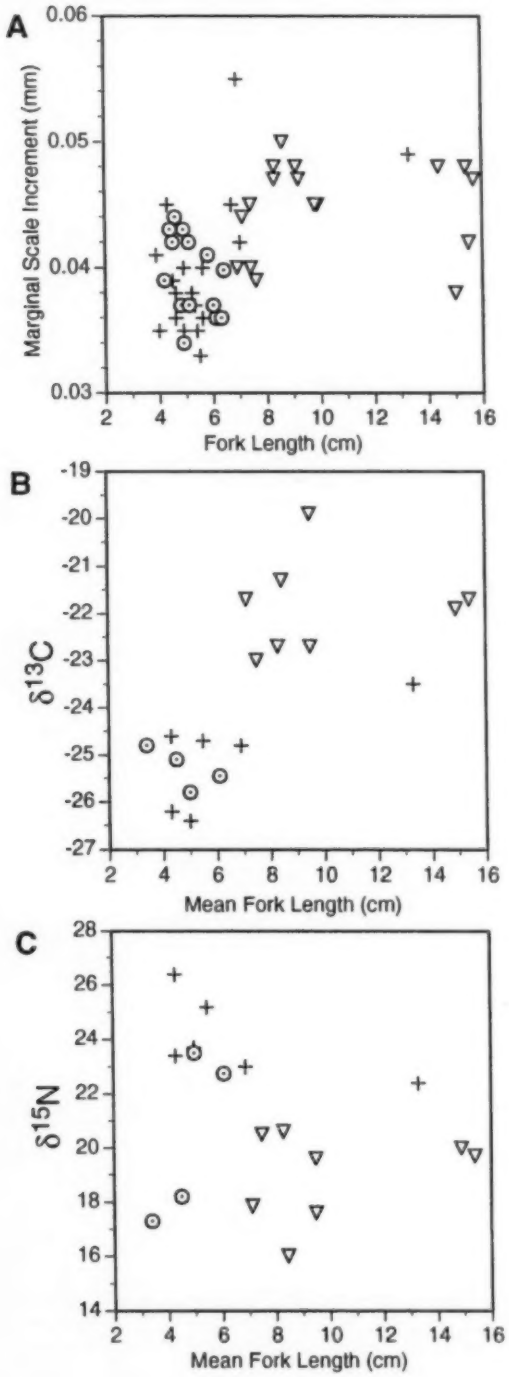
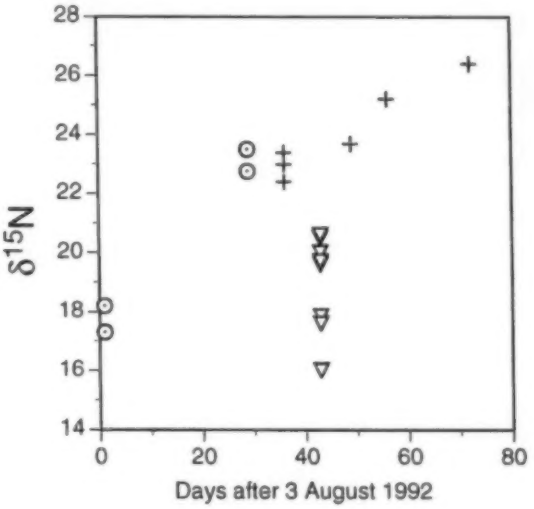


Fig. 4. Values for $\delta^{15}N$ as a function of time for the three sampled regions in the Delaware estuary. Symbols: region 1, ▽; region 2, +; region 3, ⊙. The Tinicum Island station is represented by the four uppermost crosses.



collected at all stations on all sampling dates, the $\delta^{13}C$ data from the one station that was sampled over time showed no significant time effect, arguing in favor of a consistent regional pattern. Even if spatial and temporal effects were partially confounded, when fish arrive in a new region, their feeding niche is qualitatively different from their feeding niche in the region that they just left.

Two processes by which the isotopic composition of a consumer may change to reflect a new habitat are new tissue growth and the turnover of existing tissue. The isotopic detection of either of these processes depends on an isotopically distinct diet in the new habitat. Tissue synthesized while in the new habitat will reflect the isotopic composition of that habitat; thus, a fish that doubles its weight after entering a new habitat should have an isotopic composition that is 50% determined by the new habitat due to growth alone. In addition, previous work on juvenile shrimp and birds suggests that the $\delta^{13}C$ of existing muscle tissue is replaced with a half-life of 1–3 weeks (Fry and Arnold 1982; Gleason 1986; Hobson and Clark 1992). However, Hesslein et al. (1993) found only 1.5–4% turnover during the same period in broad whitefish (*Coregonus nasus*). Using the faster estimate of isotope turnover and excluding somatic growth, one half of the isotopic composition of a consumer may reflect its diet over the most recent 1–3 weeks, while one half reflects previous diets (Hobson and Clark 1992). The processes of new tissue growth and the turnover of existing tissue work together, such that fish are naturally labeled with chemical tags that strongly reflect the region of most recent somatic growth. Our findings of regional variation in isotopic compositions indicate that juvenile striped bass spend sufficient time in a given region to acquire isotopic compositions that are distinct from one another.

Fish from region 1 had relatively positive $\delta^{13}C$ values, typical of marine phytoplankton, while fish from further upstream

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(regions 2 and 3) were significantly more negative, suggesting an influence from freshwater and (or) terrestrial vegetation.

The occurrence of a relatively positive $\delta^{13}C$ signal in juvenile striped bass collected in the oligohaline region suggests one or more of the following explanations: (*i*) estuarine circulation transports a marine or estuarine food source upstream to the oligohaline region, where it contributes to the nutrition of juvenile striped bass; (*ii*) autochthonously produced algal biomass in the oligohaline region has a marine-like $\delta^{13}C$ signal; (*iii*) organic matter of mixed origin mimics the $\delta^{13}C$ signal of marine seston (Cifuentes 1991); (*iv*) juvenile striped bass from region 1 may forage further downstream in estuarine waters; and (*v*) regional differences in $\delta^{13}C$ were influenced by lipid content (Sholto-Douglas et al. 1991).

The contribution of upstream- versus downstream-derived organic C to the diet of juvenile striped bass can be estimated using end-member $\delta^{13}C$ values for upstream plants (terrestrial C3 plants and (or) ^{13}C-depleted phytoplankton; $\delta^{13}C$ of ca. –28‰; Peterson and Fry 1987) and phytoplankton (POM) from lower Delaware Bay during summer to fall (ca. –20.5‰; Cifuentes et al. 1988). Assuming a 3‰ trophic fractionation between plant material and fish (1‰ per trophic level), upstream plants appear to fuel much of the striped bass food web in regions 2 and 3 and a smaller proportion in region 1. Thus, despite being collected in low-salinity waters, fish from region 1 derive a significant proportion of their nutrition from further downstream, consistent with explanation 1, above. However, this calculation must be considered tentative because of the high degree of variability of previously reported $\delta^{13}C$ particulate organic matter (POM) values (Cifuentes et al. 1988; Fogel et al. 1992) and our reliance on $\delta^{13}C$ POM values from previous studies.

The availability and isotopic composition of dissolved inorganic carbon ($DI^{13}C$), as well as active transport systems for concentrating DIC, may influence the $\delta^{13}C$ of phytoplankton (Fogel et al. 1992). Those authors found a strong positive relationship between salinity and $DI^{13}C$, which, other things being equal, would cause an increase in the $\delta^{13}C$ of phytoplankton downstream (explanation 2, above). However, they concluded that the uptake and fractionation of C isotopes by phytoplankton is complex and not related to any one factor. In fact, both Fogel et al. (1992) and Cifuentes et al. (1988) pointed to the high degree of variability in the isotopic composition of POM in the Delaware River estuary.

Inputs of organic matter derived from terrestrial plant material from the watershed may influence the $\delta^{13}C$ values of suspended POM (explanation 3, above). For example, terrestrially derived C3 plant material would tend to lower the $\delta^{13}C$ values of suspended POM toward –28‰, while inputs of C4 material (whether marsh-derived or agricultural) would tend to elevate $\delta^{13}C$–POM values toward –13‰ (Peterson and Fry 1987; Cifuentes 1991). Sewage is another potential allochthonous input. Cifuentes et al. (1988) did not consider any of these potential inputs to be important contributors to suspended POM in the Delaware estuary. They did, however, find an overall increase of ca. 3‰ in the $\delta^{13}C$ values of suspended POM and bottom sediments between their upstream stations (within our region 2) and stations further downstream (see also Chmura and Aharon 1995).

Although the first three potential explanations are consistent with the heavier $\delta^{13}C$ values of juvenile striped bass from region 1, the other two possibilities cannot be excluded without additional information.

The $\delta^{15}N$ values for juvenile striped bass were unusually high relative to those of marine or freshwater fish (Fig. 2C); however, this does not necessarily signify that the fish occupy a high trophic level. Trophic level is a relative term that can be estimated from the $\delta^{15}N$ difference between a consumer and the first trophic level using the empirical relationship, 1 trophic level = 3.5‰ $\delta^{15}N$ (e.g., Minagawa and Wada 1984; Fry 1988; Wainright et al. 1993). It was assumed that suspended POM, which includes phytoplankton, represents the base of the food chain (the first trophic level). Because the stable isotopic composition of POM varies seasonally and geographically (Wainright and Fry 1994), we used POM data from Cifuentes et al. (1988), which were specific to the study area and season of collection for the first trophic level ($\delta^{15}N$ = 8.3‰ and 10.0‰ in regions 1 and 2, respectively). Juvenile striped bass were estimated to occupy the third and fourth trophic level in regions 1 and 2 of the Delaware River, respectively. The POM data were unavailable to calculate trophic level in region 3. Although the isotopic composition of POM may have changed since 1984–1985 when those samples were collected (Cifuentes et al. 1988), these calculations suggest that the trophic level of juvenile striped bass may be lower in region 1 than in region 2.[3]

Although the normalization of the striped bass $\delta^{15}N$ values to those of POM seems to give a reasonable estimate of trophic level, we must still explain why the $\delta^{15}N$ of fish and POM are relatively high. Anthropogenic inputs of dissolved N in urban areas of the Delaware River (Lipschultz et al. 1986; Albert 1988) may influence the N stable isotopic composition of organic matter at the base of the food web. Sewage-derived ammonium may be incorporated into phytoplankton or bacterial biomass (either through nitrification or heterotrophic uptake), with associated isotopic fractionation (Cifuentes et al. 1988; Mariotti et al. 1984). Dissolved organic N may enter through the microbial loop. These biotic processes may lead to relatively heavy $\delta^{15}N$ values for POM. Striped bass from region 2, and downstream stations within region 3 where anthropogenic N effects are strongest (Lipschultz et al. 1986), had strikingly heavy $\delta^{15}N$ values (Fig. 2).

Nitrification has been proposed as a potential source of relatively high $\delta^{15}N$ values in plankton communities in the urbanized areas of the Delaware River (Lipschultz et al. 1986; Cifuentes et al. 1988, 1989; Fisher et al. 1988) and in other coastal areas (Mariotti et al. 1984; Wainright and Fry 1994). The $\delta^{15}N$ results from Tinicum Island (region 2; Table 1, Fig. 4) suggest that if nitrification indeed contributes to the relatively high $\delta^{15}N$ values of POM and fish, then the process may become seasonally more influential until at least October.

Implications

This study indicates that juvenile striped bass show spatial variation in both growth rate and, possibly, feeding niche

[3] Note added in proof: $\delta^{15}N$ POM data from Sept. to Oct., 1993–1994 (N. Sheats, Harvard University, unpublished data) were similar to those used in our trophic level calculations for region 1, but were higher for region 2. This confirms our estimate of trophic level for region 1, but shows that our estimate for region 2 depends on which POM data set is used.

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within the Delaware River estuary. Striped bass are highly mobile as adults and are relatively slow growing (on a weight-specific basis). Therefore, regional differences in growth rate and isotopic composition would not be expected. However, our results indicate that the more rapidly growing juveniles spend sufficient time in a region less than 20 km long to assume a distinctive isotopic signal. This association suggests (but does not prove) a causal relationship between growth rate and a qualitative aspect of the diet. The regional dietary difference appears to be divisible into at least two components: (*i*) regional variation in the trophic level of striped bass within the Delaware River and (*ii*) variation at the first trophic level that is propagated to striped bass. Food abundance alone should not affect stable isotopic compositions.

The potential role of anthropogenic inputs in determining isotopic compositions of this fish species is striking and needs to be confirmed. Furthermore, the mechanism by which anthropogenic inputs enter food webs, and affect isotopic values, i.e., dissolved inorganic N input versus dissolved organic N or particulate organic N input, should be investigated.

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Larval habitat of American brook lamprey

F.W.H. Beamish and S. Lowartz

Abstract: American brook lamprey, *Lampetra appendix*, and physical habitat information were collected during the summer from 10 second-order streams throughout much of the species' geographical distribution. Larval density, several descriptors of larval length composition, particle size distribution, hydraulic conductivity, porosity, organic content, current velocity, and water depth were measured for a number of sites within each stream. Multiple regression analyses indicated that larval density was positively related to the percentage of medium-fine sand and organic matter in the substrate. Larval density was typically high when the length range was wide and small lamprey were relatively abundant, reflecting similar habitat requirements among age-classes as well as the dynamics between larval recruitment and mortality. Three patterns were observed among the 10 streams. In three streams, larval density was strongly related to both medium-fine sand and organic matter; in two streams, increases in larval density were less noteable; and in five streams, abundance was more strongly related to medium-fine sand than organic matter. Other variables did not significantly affect larval density. The important habitat variables are discussed in the context of the species' ecology.

Résumé : On a obtenu des informations sur la lamproie de l'est, *Lampetra appendix*, et sur son habitat physique au cours de l'été, dans 10 cours d'eau du second ordre un peu partout dans l'aire de distribution géographique de cette espèce. On a mesuré la densité des larves et plusieurs descripteurs de la taille des larves, de la composition, de la distribution granulométrique, de la conductivité hydraulique, de la porosité, de la teneur en matières organiques, de la vitesse du courant et de la profondeur de l'eau, à un certain nombre d'endroits dans chaque cours d'eau. Des analyses par régression multiple indiquaient l'existence d'une corrélation positive entre la densité des larves et le pourcentage de sable mi-fin et de matières organiques dans le substrat. La densité des larves était normalement élevée lorsque la plage des tailles était grande et que les petites lamproies étaient relativement abondantes, ce qui reflète des exigences semblables relatives à l'habitat chez les catégories d'âges, ainsi que la dynamique entre le recrutement et la mortalité chez les larves. On a observé ces résultats dans 10 cours d'eau. Dans trois cours d'eau, la densité des larves était étroitement liée au sable mi-fin et aux matières organiques; dans deux cours d'eau, la densité accrue des larves était moins évidente; tandis que dans cinq cours d'eau, l'abondance était étroitement reliée au sable mi-fin et aux matières organiques. Les autres variables n'avaient aucun effet significatif sur la densité des larves. On examine les importantes variables de l'habitat dans le contexte de l'écologie de l'espèce.
[Traduit par la Rédaction]

Introduction

Adult lampreys deposit their eggs in nests constructed in the gravel substrate of riffle regions in streams. After completing the embryonic and free embryonic phases in the nest, larvae move to softer sediment. Larvae burrow by shoving their head into the sediment with vigorous contractions of the tail (Sawyer 1959; Potter et al. 1970). The anterior portion of the body enters the sediment in this manner with the posterior end ingressing more gradually using the flared oral hood as an anchor (Sterba 1962). Mucous secretions from the endostyle line the burrow and provide structural support by cementing sediment particles. Within the burrow, larvae pump water into their branchial chamber from which they obtain food and exchange respiratory gases (Hardisty and Potter 1971; Randall 1972; Potter 1980).

Most qualitative studies of larval habitat emphasize the importance of soft sediment, the presence of organic matter, low water velocity, shallow depth, and riparian cover (Schultz 1930; Enequist 1937; Hardisty 1944; Applegate 1950). Manion and McLean (1971) observed sea lamprey larvae, *Petromyzon marinus*, to be most abundant when the majority of particles in the substrate were less than 0.5 mm. An extensive examination of the habitat used by larval brook lamprey, *Lampetra planeri*, in a Swedish stream identified the proportion of coarse sand and chlorophyll in the substrate as well as water depth and velocity to be important factors (Malmqvist 1980). In perhaps the most comprehensive study of lamprey habitat, Potter et al. (1986) identified organic matter, chlorophyll *a*, macrophyte roots, and low-angle shading as important habitat characteristics for the Australian lamprey larvae, *Geotria australis*, in a single stream throughout the year. Other variables were seasonally important.

Recently, Beamish and Jebbink (1994) examined larval habitat requirements of southern brook lamprey, *Ichthyomyzon gagei*, among a wide range of streams. They found that larvae of small mean size preferred a greater proportion of fine sand in the substrate than did larvae of large mean size. The present study also examined the habitat requirements of lamprey but more critically discriminated the requirements in relation to larval size. Information was collected for larvae of American brook lamprey, *Lampetra appendix*, from 10 streams selected from throughout much of the species' geographical distribution (Lee et al. 1980; Page and Burr 1991).

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F.W.H. Beamish[1] **and S. Lowartz.** Department of Zoology and Institute of Ichthyology, University of Guelph, Guelph, ON N1G 2W1, Canada.

[1] Author to whom all correspondence should be addressed.
e-mail: bbeamish@uoguelph.ca

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Table 1. Location of the creeks sampled in the present study.

Creek	County or district	State or province
Buffalo	Carter	Tennessee
French	Chautauqua	New York
Grand	Geauga	Ohio
Macochee	Logan	Ohio
McColley Pond	Kent	Delaware
Nolichucky	Unicoi	Tennessee
Pratt Branch	Kent	Delaware
Roan	Johnson	Tennessee
Stokely	Algoma	Ontario
Tier	Algoma	Ontario

Materials and methods

American brook lamprey and substrate were collected and measurements of habitat made during the summer between June 1991 and August 1993 from 10 second-order streams in eastern North America (Table 1). A visually diverse range of sites was selected in each stream to provide information on where larvae are most and least likely to exist. Physical limitations forced most site selection along stream margins under similar conditions of cover; however, when conditions such as depth and substrate composition were suitable, sites were selected also from the centre of streams. Rooted macrophytes rarely occurred in portions of the streams sampled and were not present at any of the sites.

Site area was based on a qualitative assessment wherein no obvious changes among physical factors occurred throughout the site. Mean site area was 3.4 ± 0.3 m^2 (±95% CI, $n = 215$). After a site was selected, lampreys were collected from the substrate by electrofishing repeatedly until no animals emerged in a single pass. Voltage and pulse rates (electroshocker model ABP-2, Instrumentation Systems Center, University of Wisconsin, Madison, Wis.) were set according to stream conductivity and temperature (D. Allan, U.S. Fish and Wildlife Service, Ludington, Mich., personal communication). Electrofishing studies with these units indicate a capture efficiency for larval lampreys in the first pass in excess of 75% (D. Allan, personal communication). In this study, mean electrofishing time per site was 9.8 ± 0.9 min (range: 3–60 min). After capture, lampreys were anaesthetized (ethyl 3-aminobenzoate at 50 mg/L)and their total length was measured (±1 mm); and after recovery they were returned to the stream.

Water depth at each site was recorded (±1 cm) as the mean of a maximum, minimum, and approximate average depth measurement. Current velocity was measured near the substrate with a calibrated meter (±0.1 cm/s; Ott, C2 type meter). Two substrate samples from within each site were collected with a hand-held acrylic corer (5 cm inner diameter) to a depth of 10 ± 1 cm. Hydraulic conductivity, a measure of the capacity for water movement through an intact substrate sample, was determined on-site for each sample using the constant head method (Elrick et al. 1981). The substrate sample was then removed from a corer and placed in a plastic bag with a small amount of 5% formalin or frozen. On return to the laboratory, substrate samples were freeze-dried.

Substrate porosity, a measure of the space among particles relative to total sample volume (Lee 1989), was estimated for dried samples from the equation $P = 1 - (d/v)/D$, where P is porosity (percent void), d is the dry weight of the sample (grams), v is the total sample volume (cubic centimetres), and D is the substrate density (grams per cubic centimetre).

Each sample of dried substrate was sieved (Fritsch Laborgeratebau shaker, 10 min at amplitude 8) to determine the distribution of particle sizes by weight. Wentworth's (1922) classification of particle sizes was modified by subdividing the two smallest categories further to provide the following: gravel, >2.00 mm, very coarse sand, 1.00–2.00 mm; coarse sand, 0.50–1.00 mm; medium sand, 0.25–0.50 mm; medium-fine sand, 0.15–0.25 mm; fine sand, 0.10–0.15 mm; very fine sand, 0.05–0.10 mm; and silt–clay, <0.05 mm.

The organic content of each sample was estimated from the reduction in weight after heating at 550°C to constant weight (8 h), according to the methods of the Association of Official Analytical Chemists (1984).

Size composition of lamprey larvae from each site was represented by average total length, length range, variance, standard deviation, the smallest and largest 5th percentile, the smallest and largest 10th percentile, the smallest and largest 25th percentile, the difference in length between the 95th and 5th percentiles, and the difference in length between the 90th and 10th percentiles.

The influence of the physical descriptors of habitat on the size and abundance of American brook lamprey larvae was analyzed for the 10 streams using multiple regression analysis and a multipass procedure (SAS Institute Inc. 1989; REG procedure). An arbitrary indicator variable was included to isolate differences among streams. This allows categorical variables to be incorporated in a regression model, which is a type of analysis of covariance (ANCOVA). Each set of indicator variables uniquely identifies a stream (Draper and Smith 1981).

Results

The regression selected to identify the significant physical habitat characteristics for American brook lamprey related the logarithm of their density to their size structure, and the proportion of medium-fine sand and organic matter, although the latter was significant only as an interaction term with the length range of larvae at the lowest 10th and 25th percentiles, respectively. Differences in this relationship among the 10 streams were compared using Tukey's studentized range test (Steel and Torrie 1980). Seven significant contrasts were found, four with Buffalo Creek and three with French Creek. Buffalo and French creeks were not significantly different from one another. Because of the significant differences among some streams, it was not possible to describe the relationship by a single regression. Rather, each stream was treated as a significant variable in describing the relationship with larval density:

$$\ln(\mathrm{LD} + 0.25) = S + 2.91 \times 10^{-2} (P_5) - 5.69 \times 10^{2} (O_g) + 1.02 \times 10^{-2} (R) - 1.41 \times 10^{-2} (L_{10}) + 8.27 \times 10^{-3} (L_{25}) + 2.84 \times 10^{-3} (O_g \times L_{10}) - 1.61 \times 10^{-3} (O_g \times L_{25})$$

where LD is larval density as number per square metre, S represents the coefficient for each stream (see Table 4), P_5 is the percentage of medium-fine sand, O_g is the percentage of organic matter in the substrate, L_{10} is the total length (millimetres) at the lower 10th percentile of all larvae from a site, L_{25} is the total length (millimetres) at the lower 25th percentile of all larvae from a site, and R is the range in total length (millimetres) of all larvae from a site.

The overall regression is significant at $p = 1 \times 10^{-20}$. The variables O_g, L_{10}, and L_{25}, although not significant by themselves (see Table 5), were retained in the model because they are components of significant interaction terms. The coefficient of multiple determination, R^2, was 0.53. A formal test of the null hypothesis, residuals are normal, was not rejected.

Characteristics of the substrate within and among the 10

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Table 2. Site means (±95% CI) and ranges (in parentheses) of the density and total length of American brook lamprey larvae and habitat characteristics.

Stream	No. of sites	Lamprey density (no./m^2)	Lamprey length (mm)	Water depth (cm)	Water velocity (cm/s)	Hydraulic conductivity (μL·cm^{-2}·s^{-1})	Porosity (% void)	Organic content (% dry weight)
Buffalo	11	1.2±0.7	157±15	19±4	5±4	16±17	44±10	3.6±1.0
		(0–3.7)	(89–218)	(15–30)	(<1–84)	(<1–84)	(17–64)	(1.5–6.2)
French	14	1.5±0.9	121±10	20±5	6±4	27±21	42±7	4.0±1.8
		(0–5.4)	(29–206)	(6–32)	(5–20)	(<1–98)	(25–68)	(1.4–10.9)
Grand	28	2.0±0.6	100±5	17±4	5±2	41±11	35±2	1.3±0.3
		(0–7.9)	(23–181)	(4–41)	(<1–14)	(<1–101)	(25–50)	(0.4–3.7)
Macochee	26	2.6±0.9	150±4	25±4	9±3	72±51	49±6	5.9±1.6
		(0–7.3)	(67–227)	(13–53)	(2–28)	(<1–580)	(21–83)	(1.8–17.7)
McColley Pond	5	4.9±3.0	105±11	6±6	5±5	62±79	43±26	9.8±13.4
		(0–8.0)	(60–140)	(2–15)	(<1–12)	(3–157)	(9–64)	(0.4–27.3)
Nolichucky	20	5.7±3.4	125±3	16±3	10±4	88±54	48±5	2.4±0.7
		(0–25.3)	(45–186)	(8–25)	(1–37)	(2–355)	(32–69)	(0.3–5.0)
Pratt Branch	30	4.2±2.0	101±10	17±3	7±1	30±16	51±6	7.8±2.3
		(0–26.7)	(42–165)	(5–25)	(<1–15)	(<1–222)	(3–76)	(0.7–21.1)
Roan	21	3.2±1.6	142±3	21±4	2±1	37±20	46±3	3.7±0.7
		(0–15.6)	(75–174)	(8–55)	(<1–6)	(<1–338)	(32–61)	(1.6–7.9)
Stokely	40	5.8±2.0	71±3	17±2	8±2	55±27	31±3	1.6±0.4
		(0–23.8)	(22–172)	(8–30)	(<1–25)	(<1–338)	(15–51)	(0.7–7.3)
Tier	20	5.1±2.5	66±6	16±3	8±2	32±11	34±3	1.8±0.3
		(1.0–23.0)	(21–175)	(7–35)	(<1–17)	(1–85)	(27–48)	(1.0–3.7)

streams were as diverse as were the larval densities (Table 2). Larval densities ranged from 0 to 25/m^2, but more commonly were from 1 to 8 larvae/m^2. The statistical significance of range ($p = 0.0001$) in the model indicates an increase in larval abundance at sites where larvae with a range of lengths exist. There was, therefore, no obvious evidence of size segregation by larvae among sites. Porosity varied from under 10 to over 70% voids. Larvae were most common when porosity varied between 30 and 50% voids and were absent at the extremes (Table 2). Hydraulic conductivity ranged from less than 1 to 580 μL·cm^{-2}·s^{-1}. Again, larvae were absent from the substrate at the extremes of hydraulic conductivity. A significant relationship with larval density was not found for the rates of interstitial flow through the substrate; therefore, this term was not retained in the model.

Particle size composition of the substrate varied widely among sites (Table 3). The positive slope found relating larval density and the percentage of medium-fine sand ($p = 0.0001$) implies that density increased as the amount of this sediment increased. Medium-fine sand (0.15–0.25 mm) typically constituted 1–35% or more of the substrates' dry weight in the 10 streams. Larval density within the 10th and 25th percentile length range tended also to be positively correlated to organic content ($p = 0.0022$ and 0.0344, respectively), which in most streams ranged from <1 to <10% dry weight. In three streams, Macochee, McColley Pond, and Pratt Branch, the range in organic content was higher and in some sites represented over 20% of the dry weight. When organic content was high, it was almost always due to an abundance of small pieces of wood. A relationship between larval density and either water velocity or depth was not apparent among the streams and sites sampled; therefore, these variables were not retained in the model.

Three general patterns of change in larval density were observed. The pattern in Buffalo and French creeks was for only a slight increase in larval density as the proportions of medium-fine sand and organic matter increased (Fig. 1). The equation predicts greatest increases in larval density when the length range is wide. The rates of increase in larval density with increases in the proportion of medium-fine sand and organic matter did not differ significantly ($p > 0.05$) between Buffalo and French creeks.

In a second group of streams that included Macochee, Roan, and McColley Pond, the increase in larval density with percent medium-fine sand and organic matter was more dramatic than that in either Buffalo or French creeks. Again, this response was greatest when the larval length range was large. Thus, in the Macochee River the equation predicts an increase in larval density from approximately 1 to 3/m^2 with an increase in organic matter from 2 to 18% when the proportion of medium-fine sand represented only 1% of the substrate's dry weight (Fig. 2). With an increase in the proportion of medium-fine sand to 22%, larval density increased from about 3 to 7/m^2 as organic content increased from 2 to 18%. These responses assume a fixed larval length range of 85 mm and lengths at the lowest 10th and 25th percentiles of 110 and 130 mm, respectively, the approximate mean values for the sites in Macochee River. Significant differences were not observed ($p > 0.05$) among Macochee, Roan, and McColley Pond in the rate at which larval density increased with the proportion of medium-fine sand and organic matter.

A third pattern was observed for the remaining streams, Grand, Nolichucky, Pratt, Stokely, and Tier. In this case, larval density increased with the proportion of medium-fine sand but changed very little with the substrate's organic content. For example, in Stokely Creek the equation predicts an increase in density from just over 3 to approximately 13 larvae/m^2 with

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Table 3. Mean (±95% CI) size distribution and range (in parentheses) of substrate particles among sites within streams.

Stream	No. of sites	Substrate particle distribution, % dry weight							
		P_1, >2.00	P_2, 1.00–2.00	P_3, 0.50–1.00	P_4, 0.25–0.50	P_5, 0.15–0.25	P_6, 0.10–0.15	P_7, 0.05–0.10	P_8, <0.05
Buffalo	11	5.9±7.4	2.7±2.5	7.4±3.4	27.8±6.4	23.5±4.7	12.6±5.4	13.6±4.1	6.4±2.4
		(0.4–37.9)	(0.1–13.5)	(0.6–19.6)	(12.3–43.7)	(8.2–35.3)	(4.5–24.5)	(4.4–23.0)	(1.9–12.5)
French	14	21.0±12.4	9.7±7.8	10.3±3.2	23.2±7.6	14.0±4.7	7.1±3.1	11.0±4.8	4.1±1.9
		(0.7–67.9)	(1.0–9.8)	(3.2–21.8)	(6.0–44.3)	(1.2–31.8)	(0.5–17.6)	(1.6–32.7)	(0.9–13.4)
Grand	28	7.5±3.7	6.7±2.2	18.9±4.2	47.3±5.9	12.5±3.7	3.3±1.7	2.7±1.2	1.1±0.5
		(<0.1–29.1)	(0.1–22.8)	(2.2–38.8)	(16.6–66.8)	(2.3–32.7)	(0.2–14.9)	(0.4–11.1)	(0.3–4.9)
Macochee	26	31.0±9.5	9.2±1.8	14.7±4.0	18.1±4.1	9.9±2.6	5.0±1.6	7.9±2.4	4.1±1.2
		(2.4–76.5)	(3.7–21.6)	(3.0–42.4)	(3.9–41.6)	(1.2–24.4)	(0.3–14.3)	(0.9–21.1)	(0.7–9.1)
McColley Pond	5	3.3±3.3	3.9±3.1	17.0±9.8	43.3±20.1	18.2±9.1	5.4±7.5	5.8±7.4	2.8±3.3
		(0.6–65.0)	(0.8–7.4)	(5.2–27.8)	(19.2–60.5)	(7.1–34.0)	(0.5–15.8)	(0.2–14.6)	(0.1–6.4)
Nolichucky	20	9.0±6.7	12.0±6.4	21.3±9.4	19.7±4.4	18.4±6.5	9.2±3.5	8.5±3.1	2.3±0.8
		(0.3–46.1)	(0.1–40.2)	(1.7–84.9)	(5.6–36.7)	(0.1–41.0)	(<0.1–24.5)	(0–16.5)	(<0.1–5.1)
Pratt Branch	30	6.0±3.8	5.2±1.3	20.6±4.9	43.0±4.4	13.9±3.4	3.2±1.0	4.2±1.4	3.8±1.2
		(0.5–46.3)	(1.0–11.8)	(2.4–56.6)	(21.8–70.7)	(1.0–38.5)	(0.1–11.0)	(0.2–16.0)	(0.2–13.1)
Roan	21	6.8±3.5	4.2±1.7	13.0±3.6	40.3±4.9	20.7±2.9	6.6±1.2	6.0±1.4	2.4±0.7
		(0–30.8)	(0.3–13.6)	(1.5–46.4)	(16.3–64.8)	(4.2–34.4)	(2.7–12.7)	(1.5–11.9)	(0.6–6.3)
Stokely	40	16.2±7.7	4.8±2.3	10.1±3.5	27.0±4.8	21.6±3.9	9.1±2.4	8.3±2.6	3.3±1.1
		(0–81.4)	(0.1–23.7)	(0.2–42.2)	(5.9–57.4)	(1.5–46.2)	(0.2–25.6)	(0.3–28.9)	(0.1–11.9)
Tier	20	12.0±5.8	11.2±3.4	22.7±5.6	31.8±5.7	13.5±4.9	4.5±1.6	3.2±1.2	1.1±0.3
		(0.5–46.3)	(0.7–25.5)	(2.6–51.4)	(13.6–48.4)	(2.0–36.2)	(0.8–10.9)	(0.8–8.6)	(0.3–2.5)

Note: Particles were classified into groups P_1 to P_8 on the basis of size (in millimetres).

Table 4. Coefficients for each stream to be applied to the regression described in the text.

Stream	Coefficient ($\times 10^{-1}$)
Buffalo	–5.77
French	–4.60
Grand	2.79
Macochee	0.09
McColley Pond	8.46
Nolichucky	7.59
Pratt	3.83
Roan	4.54
Stokely	2.53
Tier	2.29

Table 5. Regression coefficients, F values, and levels of significance (p) for variables in the multiple regression.

Variable	Regression coefficient	F	p
S		4.56	
P_5	2.91×10^{-2}	24.70	0.0001
O_g	-5.69×10^{-2}	2.02	0.1572
R	1.02×10^{-2}	40.61	0.0001
L_{10}	-1.41×10^{-2}	3.62	0.0589
L_{25}	8.27×10^{-3}	1.40	0.2383
$O_g\times L_{10}$	2.84×10^{-3}	9.66	0.0022
$O_g\times L_{25}$	-1.61×10^{-3}	4.55	0.0344

Note: The coefficients for each stream, S, are given in Table 4. Stream differences are referred to in the text.

an increase in medium-fine sand from 2 to 46%, when the organic content was 0.5% of dry weight, larval length range was 129 mm, and the lower 10th and 25th percentiles were 70 and 80 mm, respectively (Fig. 3). These characteristics represented averages from the 20% of the sites with the widest length range. When organic content was 6.5% under otherwise similar conditions, density increased from almost 4 to approximately 14 larvae/m^2, only a slight elevation over that predicted for an organic content of 0.5%. The response to the proportions of medium-fine sand and organic matter was similar when larvae were more homogeneous in length; however, densities were much lower (Fig. 3). Tukey's test revealed no significant differences ($p > 0.05$) among the five streams in the rate of increase in larval density, for similar length characteristics, with increases in the proportion of medium-fine sand and organic matter. Overall, significant differences were found in the rate of increase in larval density between streams described by the first and second patterns and between streams described by the first and third patterns.

Lamprey density at a site was highest in all streams when the length range was wide and small larvae were most abundant. In Pratt Branch, when the substrate's organic matter and medium-fine sand were 10 and 25%, respectively, the length range was 100 mm, and the length at the lower 10th percentile was 30 mm, the equation predicts a decline from 2.3 to 1.2 larvae/m^2 when the length at the 25th percentile increased from 35 to 60 mm. Under the same conditions, except for larval lengths of 60 and 65 mm at the 10th and 25th percentiles, respectively, density was estimated at only 0.3/m^2. In Macochee River when the substrate's organic matter and medium-fine sand were 4 and 15%, respectively, the larval length range was 60 mm, and the length at the 10th percentile was 40 mm, density declined from 2.7 to 1.7 when the length at the 25th percentile increased from 50 to 80 mm. For similar

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Fig. 1. Larval density in Buffalo Creek in relation to the proportions of medium-fine sand and organic matter, both expressed as percent dry weight of substrate. Length characteristics of larvae were fixed at a range of 50 mm and the lengths at the lowest 10th and 25th percentiles, 130 and 135 mm, respectively. These length characteristics represented the approximate mean values for the sites in Buffalo Creek.

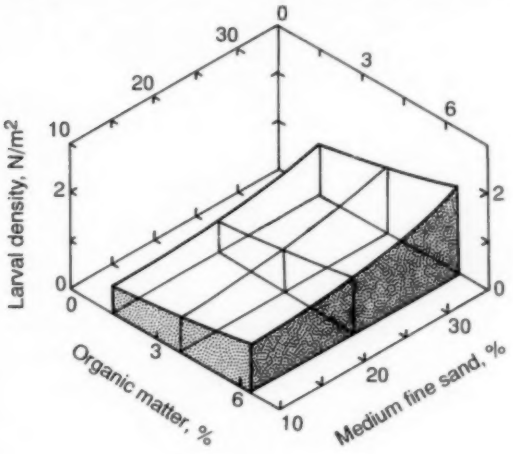
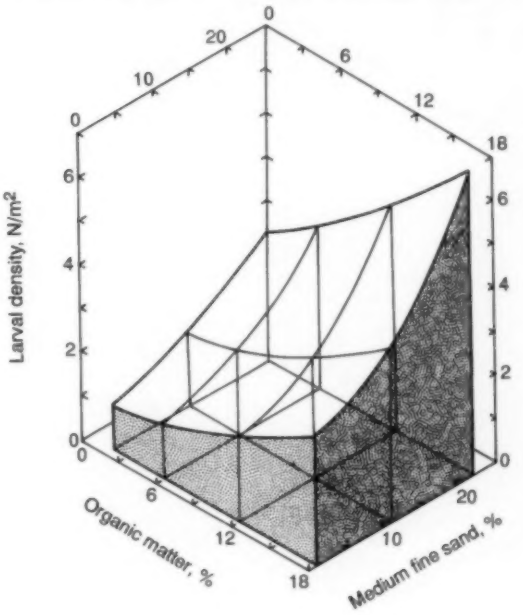


Fig. 2. Larval density in Macochee River in relation to the proportions of medium-fine sand and organic matter, both expressed as percent dry weight of substrate. Length characteristics of larvae were fixed at a range of 85 mm and the lengths at the lowest 10th and 25th percentiles, 110 and 130 mm, respectively. These length characteristics represented the approximate mean values for the sites in Macochee River.



conditions except for lengths at the 10th and 25th percentiles of 60 and 80 mm, density was estimated at 1.6 larvae/m^2.

Discussion

The soft sediment in streams protects larval lampreys from predators, but it must also provide for burrow construction and water exchange. The detritivorous larvae depend on a unidirectional flow of water through their branchial chamber for the provision of food and exchange of respiratory gases and metabolic wastes (Hardisty and Potter 1971). The high affinity of lamprey blood for oxygen is important for survival within a substrate where the oxygen concentration may be far below that in the water column (Manwell 1963; Potter et al. 1970). This study, conducted during the summer, suggests a similarity in the physical habitat preferred by American brook lamprey of all sizes throughout their geographical distribution. Larval density was typically highest when the length range was wide and small larvae were abundant, reflecting also the dynamics between larval recruitment and mortality. Of the descriptors of habitat evaluated in the present study, only the proportion of medium-fine sand and, to a lesser extent, organic matter were significant.

The significance of organic matter was much greater for some populations of American brook lamprey than others. In the Macochee, Roan, and McColley Pond, larval density increased considerably with organic matter. In the other streams, lamprey abundance was much less influenced by the organic content of the substrate. Beamish and Jebbink (1994) found substrate organic content to significantly affect abundance of southern brook lamprey. In sharp contrast, Malmqvist (1980) did not find organic matter to be a significant descriptor of brook lamprey habitat, but he did find chlorophyll *a* concentration to be important. Potter et al. (1986) found organic matter and chlorophyll *a* abundance to be directly related to the density of larval Australian lamprey throughout the year in a southwestern Australian stream. Potter et al. (1986) and Malmqvist (1980) measured organic content in only the upper 20 and 30 mm of the substrate profile whereas in this study and that by Beamish and Jebbink (1994) it was measured within the upper 100 mm. There is no doubt that organic matter is critical to the well-being of lamprey larvae. Sutton (1993) found organic detritus to represent almost 98% of the diet of larval sea and northern brook lamprey, *Ichthyomyzon fossor*, with algae contributing about 2% and bacteria, 0.1%. The organic detritus was composed largely of particles of biofilm, presumably fragmented from the periphyton community. It is not particularly surprising that Malmqvist (1980) found the concentration of chlorophyll *a* and not organic matter to be significant. Chlorophyll *a* may be a good indicator of the availability of biofilm as algae along with bacteria manufacture the noncellular organic material on which lamprey feed (Sutton 1993). The presence of other organic matter may be of little consequence to larvae.

Sutton (1993) found some seasonal variation in the consumption of detritus but a reasonable average was about 5 mg·g^{-1}·day^{-1}. Assuming a similar feeding regimen by American brook lamprey and a larval density equivalent to 5 g/m^2, the daily requirement of organic detritus is about 25 mg. This represented a percentage of between approximately 0.32 $\times$ 10^{-4} and 0.74 $\times$ 10^{-4} by weight of the upper 10 cm of the

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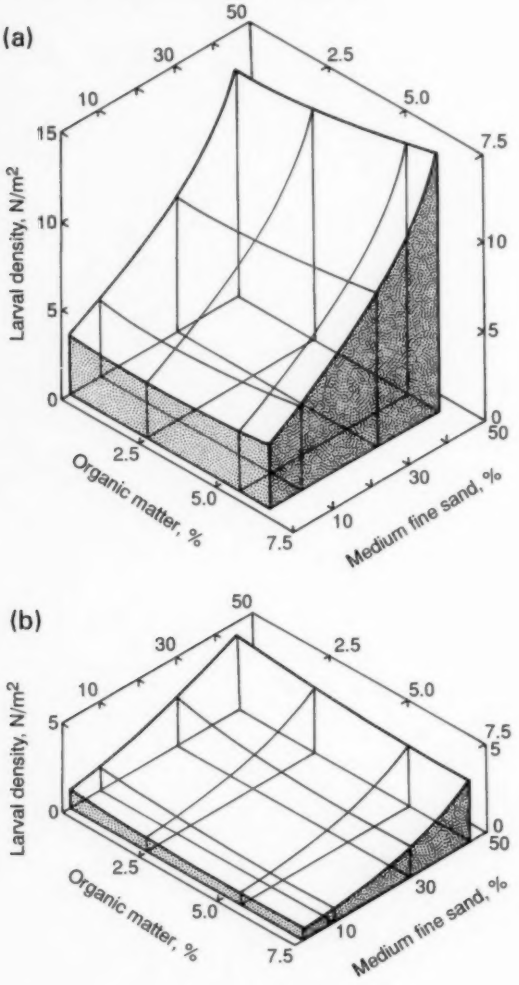
Fig. 3. Larval density in Stokely Creek in relation to the proportions of medium-fine sand and organic matter, both expressed as percent dry weight of substrate. (*a*) Length characteristics were fixed at a range of 129 mm and the lowest 10th and 25th percentiles, 70 and 80 mm. These characteristics represent averages from those 20% of the sites with the widest length range. (*b*) Length characteristics were fixed at a range of 5 mm and the lowest 10th and 25th percentiles, 26 and 28 mm. These characteristics represent averages from those 20% of the sites with the most narrow length range.



substrate and certainly much lower quantities of organic matter than were found at any of the sites. Much of the organic matter in the samples collected consisted of wood pieces and leaves from riparian vegetation and was obviously much too large to be ingested by larvae. If, as seems likely, larvae feed largely near the substrate surface on dislodged fragments of biofilm, the significance of organic matter within the substrate may have relatively little direct dietary significance. Decomposing organic matter may, however, be important to the interstitial movement of water through the sediment.

Particle size composition of the substrate has been identified frequently as being important for lamprey larvae (Hardisty and Potter 1971). Larvae burrow by pushing their unprotected head into the sediment with vigorous contractions of the tail. Once within the sediment the larva must allow for burrow construction and water movement. Very small particles such as clay can compact, forming an inpenetratable barrier to both larvae and water. An overabundance of silt and clay particles is also likely to inhibit oxygen uptake by clogging gill lamellae and dermal pores across which gaseous exchange occurs. Large particles are simply too heavy for larvae to dislodge. Qualitative and quantitative studies emphasize a direct correlation between larval abundance and that of particles less than about 0.5 mm in diameter (Schroll 1959; Manion and McLain 1971; Potter et al. 1986; Beamish and Jebbink 1994). Schroll (1959) identified the important size fraction as being 0.18–0.38 mm whereas Potter et al. (1986) found it to be 0.2–0.6 mm. Manion and McLain (1971) observed sea lamprey larvae to be most abundant when the majority of particles were less than 0.5 mm in diameter. In a laboratory study, Lee (1989) tested sediments composed solely of silt–clay (≤0.063 mm) and coarse sand (0.5–1.0 mm) and found that neither was selected by either sea or American brook lamprey larvae. Fine (0.125 mm) and medium (0.25–0.5 mm) sand were the preferred sediment sizes, similar to the observations in the present study. The extent to which studies have discriminated particle size may at least partially explain the small variation in optimal size. Most studies have examined habitat in only one stream where a full range of particle size compositions may have been available at comparatively few sites. It is, of course, also possible that differences in preference do occur among species. The abundance of American brook lamprey larvae is predicted to increase exponentially with the amount of medium-fine sand, which in this study ranged from <0.1 to 46% by weight. The extent to which this relationship might be extrapolated and the roles of other particle sizes are not known. Beamish and Jebbink (1994) found that fine and coarse sand provided the optimal combination of particle sizes when these substrates represented at least 40 and 8%, respectively, of the substrate inhabited by southern brook lamprey.

Optimal particle size and abundance for American brook lamprey did not change with larval length. This appears to be the first study to consider larval length. Very small larvae (under 25 mm) were seldom captured in this study. Typically, after completing the free embryonic phases of development in the nest, young larvae move to areas of low flow and small particles, with the latter largely composed of organic matter. Within a few months, larvae move from these nursery areas to join the remainder of the population. The observation that neither hydraulic conductivity nor porosity of the sediments was a significant descriptor of larval habitat should not be interpreted to mean that flow through the sediments is not important. Clearly it is; however, the results of this study indicate that in a lotic environment, particle size composition is a much stronger indicator of habitat suitability. Lee (1989) found that in the lentic environment substrate hydraulic conductivity was a significant determinant of larval habitat.

Several other important characteristics of larval lamprey habitat have been identified that were not found to be significant in this study, including current velocity and the presence

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of eddies (Hardisty 1944; Applegate 1950; Schroll 1959; Potter et al. 1986). Sites characterized by eddies were not examined in the present study, but our casual observations indicated these areas as being favorable larval habitat in accord with the findings of others. Current velocity was measured and not found to be a significant variable even though it frequently was <1 cm/s. Particle size distribution in the lotic environment is associated closely with current velocity, particularly during periods of high discharge. During summer, discharge, and hence current velocities, tend to be low and the substrate stable. Velocity is more likely to be important during periods of high flow and substrate instability.

Water depth was not found to be a significant variable in this study, in contrast to observations made in some earlier studies. Thus, Potter et al. (1986) found abundance of the Australian lamprey to vary inversely with depth, during both summer and winter when discharge is often low and quite stable. In the present study the water was relatively shallow, with most sites less than 30 cm deep. Another variable sometimes reported to be important but not identified in this study is the presence of macrophyte roots (Hardisty 1944; Applegate 1950; Thomas 1962; Potter 1970). Macrophytes were virtually absent in all of the streams examined in this study and hence were not considered. Life within the substrate affords the photophobic lamprey larvae protection from light. Malmqvist (1980) did not find external shading of solar radiation to improve otherwise suitable larval habitat. In contrast, Potter et al. (1986) found that reductions in both light intensity and low-angle shading improved larval habitat. Sites in the present study were mainly along the margins of streams and afforded at least moderate cover by streamside vegetation.

This study found American brook lamprey larvae of virtually all sizes to have similar habitat preferences among streams throughout the species' geographical range. The most important factor is the abundance of medium-fine sand with organic matter also being important, particularly in some streams. Medium-fine sand appears to be ideal for burrowing and burrow construction. As well, it allows for an adequate flow of water through the branchial chamber and over the body to meet the animal's nutritional needs and to allow for the exchange of respiratory gases and metabolic wastes.

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Respiratory and acid–base disturbances in rainbow trout (*Oncorhynchus mykiss*) blood during exposure to chloramine T, paratoluenesulphonamide, and hypochlorite

Mark D. Powell and Steve F. Perry

Abstract: The effects of acute exposure to chloramine T, a common aquaculture therapeutic agent, and its degradation products sodium hypochlorite and paratoluenesulphonamide (*p*TSA) on the respiratory and acid–base physiology of rainbow trout (*Oncorhynchus mykiss*) were examined using an extracorporeal circulation. Fish in which the coeliac artery was fitted with indwelling polyethylene catheters were exposed to either 2 or 9 mg·L^{-1} chloramine T (active ingredient), 0.45 mg·L^{-1} sodium hypochlorite, or 9 mg·L^{-1} *p*TSA. Control fish were not exposed to any chemical. There was a significant increase in ventilation frequency and arterial P_{CO_2} with chloramine T and hypochlorite treatments. A significant acidosis was evident in fish exposed to hypochlorite but not chloramine T. Chloramine T exposure had no effect on arterial P_{O_2}, which was significantly reduced in fish exposed to hypochlorite. There was no significant effect of *p*TSA exposure on any of the variables measured. The respiratory changes may be partially explained by changes in the gill gas transfer conductance owing to the secretion of mucus onto the gill surface.

Résumé : Grâce à la circulation extra-corporelle, on a examiné les effets d'une exposition aiguë à la chloramine T, un agent thérapeutique couramment employé en aquaculture, et à ses produits de dégradation, soit l'hypochlorite de sodium et le *para*-toluènesulfonamide (*p*TSA), sur la physiologie de la respiration et de l'équilibre acide–base chez la truite arc-en-ciel (*Oncorhynchus myskiss*). Des poissons dont le tronc coeliaque avait été muni d'une sonde à demeure en polyéthylène ont été exposés à 2 ou à 9 mg·L^{-1} de chloramine T (ingrédient actif) ou à 0,45 mg·L^{-1} d'hypochlorite de sodium ou 9 mg·L^{-1} de *p*TSA. Les poissons témoins n'ont été exposés à aucun produit chimique. Il y avait augmentation notable de la fréquence de ventilation et de la P_{CO_2} artérielle chez les poissons traités avec de la chloramine T et de l'hypochlorite. Il y avait des signes d'une importante acidose chez les poissons exposés à l'hypochlorite mais non chez ceux exposés à la chloramine T. La chloramine T n'exerçait aucun effet sur la P_{O_2} artérielle qui était considérablement réduite chez les poissons exposés à l'hypochlorite. L'exposition au *p*TSA ne produisait aucun effet significatif sur les variables mesurées. On peut partiellement expliquer les changements respiratoires par des changements de conductance de transfert des gaz dans les branchies, causés par la sécrétion de mucus sur la surface des branchies.
[Traduit par la Rédaction]

Introduction

Chloramine T (*n*-sodium-*n*-chloroparatoluenesulphonamide) use in aquaculture facilities in North America is widespread and common. Although not licensed for use with fish as a therapeutic agent, it is frequently used as an effective treatment of bacterial gill diseases at concentrations ranging from 6.5 to 10.0 mg·L^{-1} (From 1980; Bullock et al. 1991) and as a preventative, prophylactic, and disinfective treatment in many freshwater hatcheries (Thorburn and Moccia 1993; Smith et al. 1993). However, despite widespread use as a disinfectant, relatively little is known about the mechanism of action of chloramine T or any physiological consequences of its treatment on fish.

Chloramine T liberates chlorine in water by nucleophilic substitution, forming the hypochlorite ion (OCl^-; Booth and McDonald 1988). Chloramine T toxicity may be dependent upon this chemical reaction, since acute toxicity (LC_{50}) is enhanced at elevated temperature, at low pH, and in soft water (Bills et al. 1988). Chloramine T and its degradation product paratoluenesulphonamide (*p*TSA) are taken up across the gill, but both are rapidly eliminated (Dawson and Gingerich 1993). However, the primary use of chloramine T is as a bactericide, which arises from the disinfective albeit irritating action of the hypochlorite ion.

Chlorine in the form of hypochlorite or the more stable mono- or di-chloramine form are highly toxic to fish, particularly salmonids (Brooks and Seegert 1977; Larson et al. 1977*a*, 1977*b*; Johnson et al. 1977; Seegert and Brooks 1978; Seegert et al. 1979; Stober et al. 1980; Brooks and Bartos 1984). Bass et al. (1977) demonstrated that sublethal chlorine pulses (0.4 and 0.5 mg·L^{-1} total residual chlorine) caused an acute hypertrophy and hyperplasia of rainbow trout branchial

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M.D. Powell[1] and S.F. Perry. Department of Biology, University of Ottawa, 30 Marie Curie, Ottawa, ON K1N 6N5, Canada.

[1] Author to whom correspondence should be sent at the following address: Department of Anatomy and Physiology, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, PE C1A 4P3, Canada. e-mail: MPOWELL@upei.ca

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epithelium and extensive mucus secretion. The same fish showed reduced arterial P_{O_2}, marked bradycardia, and hyperventilation (Bass and Heath 1977). In a recent study (Powell et al. 1994), repeated intermittent exposure of rainbow trout to chloramine T at therapeutic concentrations caused behavioral changes consistent with respiratory distress (i.e., fish crowded at the tank surface and appeared to hyperventilate). The same fish showed an apparent chloride cell hyperplasia and other morphological changes (increase in expression of the chloride cell apical plasma membrane) of their gills (Powell et al. 1995) similar to that seen during compensation of acid–base disturbances in freshwater fish (e.g., metabolic alkalosis) (Goss et al. 1992*a*, 1992*b*; Goss and Perry 1993).

The present study was designed to assess the effects of a single pulse of chloramine T at therapeutic concentrations and its primary degradation products, hypochlorite and *p*TSA, on respiratory and acid–base variables of rainbow trout in vivo.

Materials and methods

Fish

The rainbow trout (*Oncorhynchus mykiss* Walbaum) used in this study were obtained from a commercial trout farm (Linwood Acres Trout Farm, Campbellcroft, Ont.) and acclimated to laboratory conditions for at least 2 weeks prior to use. Throughout the acclimation period and in subsequent experiments the fish were held under natural photoperiod in flowing, aerated, and carbon-filtered dechlorinated City of Ottawa water (<0.03 mg·L^{-1} residual chlorine as determined by the DPD method; Franson 1978) at 10°C containing 118.9 ± 2.4 (mean ± SE) μM Na^+, 151.8 ± 0.9 μM Cl^-, 365.8 ± 8.2 μM Ca^{2+}, and 19.3 ± 0.5 μM K^+ at pH 6.8.

Surgical procedures

Adult trout of mean mass 670.7 ± 15.8 g were anaesthetized with tricaine methanesulphonate (MS–222) (100 mg·L^{-1}), and the gills constantly irrigated with the same oxygenated anaesthetic solution throughout the following surgical procedures. A polyethylene (PE–50; Clay Adams Inc., Becton Dickinson and Company, Sparks, MD 21152-0370) catheter was implanted in the dorsal aorta according to the method of Soivio et al. (1975). Either the operculum was catheterized by drilling a small hole through the opercular bone approximately 1 cm from the trailing edge and inserting through the hole a 50-cm length of PE–160 tubing heat flared at one end, or 1-cm^2 brass plate electrodes were sutured to the epithelium of each operculum. An incision was then made into the right flank just posterior to the head and the coeliac artery was isolated and catheterized with PE–50 in both orthograde and retrograde directions (Thomas and LeRuz 1982). The wound was closed and the animal allowed to recover in fresh water in a black acrylic respirometer box with flowing water. All catheters were flushed with Cortland's saline (Wolf 1963) prior to insertion. Following at least 1 day of recovery, the catheters were flushed with heparinized Cortland's saline (250 IU ammonium heparin; Sigma Chemical Company, St. Louis, Mo.) prior to experimentation.

In a second set of experiments, rainbow trout of mean mass 206.5 ± 7.5 g were anaesthetized as above and PE–50 catheters implanted into the dorsal aorta according to the method of Soivio et al. (1975). These fish were placed in individual black acrylic boxes with flowing fresh water and allowed to recover for 18–24 h prior to chemical exposure.

Blood measurements

Extracorporeal circulation

Arterial P_{O_2}, P_{CO_2}, and pH were measured continuously by drawing blood by peristaltic pump (flow rate 0.45 mL·min^{-1}) from the coeliac artery and passing it over temperature-controlled O_2 (Radiometer E5046), CO_2 (Radiometer E5036), and pH (Metrohm) electrodes arranged in series and connected to a Radiometer PHM 73 acid–base analyzer. The blood was then returned to the coeliac artery. Dorsal aortic blood pressure and opercular pressure were monitored using Bell and Howell type 4–327 I pressure transducers (Bell and Howell, Pasadena, Calif.) connected to a recording physiograph (Lafayette). Opercular impedance was monitored by means of a customized impedance converter and amplifier. Analog outputs from the acid–base analyzer, physiograph, and impedance converter were transformed into digital outputs using an analog–digital interface (Data Translation Inc.) and relayed to a microcomputer. The output was recorded using customized data acquisition software (written by P. Thören, Göteborg, Sweden). Ventilation frequency was measured visually by counting buccal movements.

Plasma bicarbonate concentrations ([HCO^{3-}]) were calculated from rearrangement of the Henderson–Hasselbalch equation (see below) using pH_a and arterial P_{CO_2} values measured with the extracorporeal circulation and constants from Boutilier et al. (1984). The nonbicarbonate buffer values for true plasma were calculated according to the regression equation given by Wood et al. (1982). Rearranging the Henderson–Hasselbalch equation,

$$(1)\quad \mathrm{pH} = \mathrm{pK} + \log_{10}\frac{\text{total } CO_2 - (\alpha_{CO_2} \times P_{CO_2})}{\alpha_{CO_2} \times P_{CO_2}}$$

gives

$$(2)\quad \text{total } CO_2 = (\text{antilog } (\mathrm{pH} - \mathrm{pK}) + 1)(\alpha_{CO_2} \times P_{CO_2})$$

and

$$(3)\quad [HCO_3^-] = \text{total } CO_2 - (\alpha_{CO_2} \times P_{CO_2}).$$

Fish were monitored in a stable resting state for 30 min prior to chemical exposure. Fish were exposed to a 60-min pulse of either chloramine T (9 mg·L^{-1} active ingredient (a.i.), n = 7) (BDH Chemicals Inc., Toronto, Ont., analytical grade lot No. 106295/15998), paratoluenesulphonamide (*p*TSA) (9 mg·L^{-1} a.i., n = 6) (Sigma, lot No. 23H3595) dissolved in 0.0012% (v/v, final concentration) dimethylsulphoxide (BDH Chemicals Inc.), sodium hypochlorite solution (0.45 mg·L^{-1}, n = 7) (BDH Chemicals Inc., Mississauga, Ont.), or water (controls, n = 5). A stock solution of each chemical was made up using the same dechlorinated water as used in the fish holding system and metered into the inflowing water of the respirometer box via a Manostat Varistaltic AL series peristaltic pump (Manostat Inc., New York) to yield the appropriate exposure concentration. Following exposure, fish were further monitored for an additional 90 min prior to termination of the experiment. Each fish received only one treatment chemical and treatments were conducted in randomized blocks.

Dorsal aorta cannulated fish

Prior to experimentation a 650-μL blood sample was withdrawn from each fish. From this blood sample, haematocrit was determined in duplicate using microcapillary tubes centrifuged at 10 000 × *g* for 10 min, haemoglobin content was determined spectrophotometrically using a commercial kit (Sigma), and total oxygen content (Ca_{O_2}) was determined according to the method of Tucker (1967). The remaining blood was centrifuged at 10 000 × *g* for 30 s, and the plasma was frozen in liquid nitrogen and stored at –80°C for later determination of catecholamine levels. Catecholamines were extracted from the thawed plasma samples according to the method of Woodward (1982) and analyzed using high performance liquid chromatography (Varian 9000 series system with electrochemical detection with an EG & G model 400 electrochemical detector) using DHBA (3,4-dihydroxybenzylamine, Sigma) as an internal standard. Further blood samples were withdrawn immediately after exposure to a 60-min pulse of each chemical (chloramine T at 9 mg·L^{-1} a.i., n = 8; paratoluenesulphonamide at 9 mg·L^{-1} a.i., n = 6; sodium hypochlorite at

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0.45 mg·L^{-1}, $n = 6$; or control, $n = 7$) and a subsequent 650-μL blood sample was withdrawn and analyzed as above following a further 60 min of recovery in fresh water.

Data manipulation and statistical analysis

For the purposes of comparing the responses of each measured variable to chemical treatment, a mean normalization transformation was used to subtract the mean base line from each successive data point. The end result is data expressed as a net change in each variable without violating assumptions of normality (Glantz 1987). The data points shown in the figures represent means at 15-min intervals ± standard error. As a consequence of this transformation, means were statistically compared with base-line values using a repeated measures analysis of variance. Post hoc analysis was performed with multiple Bonferroni-corrected t tests (Glantz 1987). Differences in the other measured blood parameters (e.g., Cao_2, haematocrit) were analyzed by repeated measures analysis of variance and differences were isolated using Bonferroni-corrected t tests. P values of 0.05 or less were considered to be significant.

Results

Ventilation frequency

Chloramine T exposure (9 and 2 mg·L^{-1}) significantly increased V_f (Figs. 1 and 2). Ventilation frequency (V_f) was also significantly elevated during exposure to sodium hypochlorite (Fig. 3), but there was no significant effect of pTSA exposure on V_f (Fig. 4). Following the withdrawal of chloramine T, V_f returned to base-line and control values within 15 min (2 mg·L^{-1} chloramine T, Fig. 2) or within 90 min (9 mg·L^{-1} chloramine T, Fig. 1). However, V_f remained significantly elevated in fish exposed to 0.45 mg·L^{-1} sodium hypochlorite (Fig. 3).

Arterial Pco_2

Both concentrations of chloramine T and sodium hypochlorite resulted in a significant increase in Pco_2 ($Paco_2$) during the exposure period (Figs. 1–3). At 9 mg·L^{-1} chloramine T, $Paco_2$ declined to base-line levels within 90 min of withdrawal of chloramine T (Fig. 1). However, at 2 mg·L^{-1} chloramine T, $Paco_2$ levels remained significantly elevated upon withdrawal of chloramine T (Fig. 2). Upon withdrawal of sodium hypochlorite, $Paco_2$ levels remained significantly elevated during the 90-min post-exposure period (Fig. 3). There was no significant change in the $Paco_2$ levels in the blood of control fish or fish exposed to pTSA (Fig. 4).

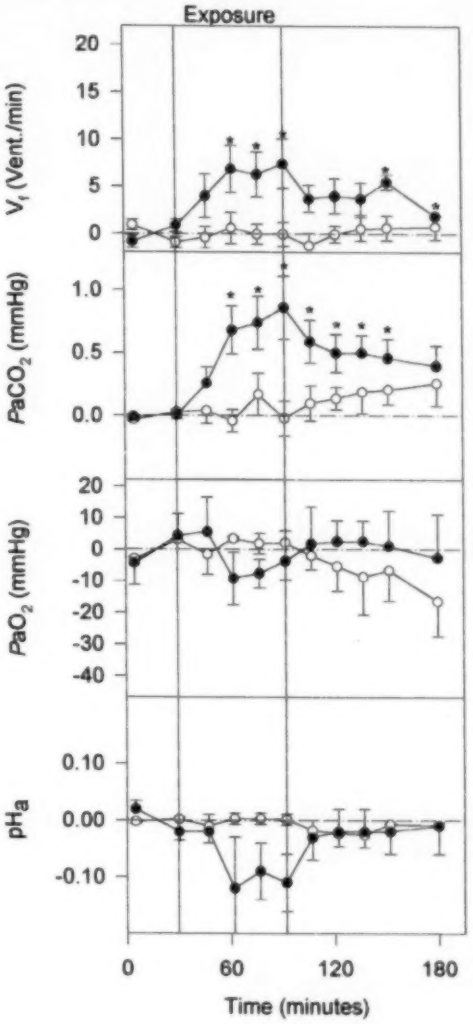
Arterial Po_2

There was no significant effect of chloramine T or pTSA exposure on the Po_2 (Pao_2) levels (Figs. 1, 2, and 4). However, exposure to 0.45 mg·L^{-1} sodium hypochlorite resulted in a significant decrease in Pao_2 that did not recover during the 90-min post-exposure period (Fig. 3). There was no significant change in the Pao_2 of control fish over the experimental period.

Arterial pH

Although fish exposed to chloramine T (at either concentration) appeared to show a decrease in arterial pH (pH_a) (Figs. 1 and 2), this was not statistically significant. However, fish exposed to 0.45 mg· L^{-1} sodium hypochlorite showed a significant decrease in pH_a during the exposure period that gradually recovered to pre-exposure values by the end of the experimental period (Fig. 3). Fish exposed to pTSA showed a gradual decrease in pH_a during the experimental period and this was pronounced throughout the recovery period (Fig. 4).

Fig. 1. The change in ventilation frequency (V_f), arterial Pco_2 ($Paco_2$), arterial Po_2 (Pao_2), and arterial pH (pH_a) during exposure to 9 mg·L^{-1} chloramine T (a.i.) (●). The chemical exposure period is represented between the two vertical bars. ○, control fish; *, significantly different from base-line zero. Error bars represent 1 SE.



Other blood parameters

There were no significant differences between the pre-exposure values for any of the treatments with the exception of mean cellular haemoglobin concentration. However, the relevant comparisons are within treatment rather than between treatment groups (i.e., pre-exposure values compared with post-exposure values for the same treatment). There was no consistent

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Fig. 2. The change in ventilation frequency (V_f), arterial P_{CO_2} (Pa_{CO_2}), arterial P_{O_2} (Pa_{O_2}), and arterial pH (pH_a) during exposure to 2 mg·L^{-1} chloramine T (a.i.) (●). The chemical exposure period is represented between the two vertical bars. ○, control fish; *, significantly different from base-line zero. Error bars represent 1 SE.

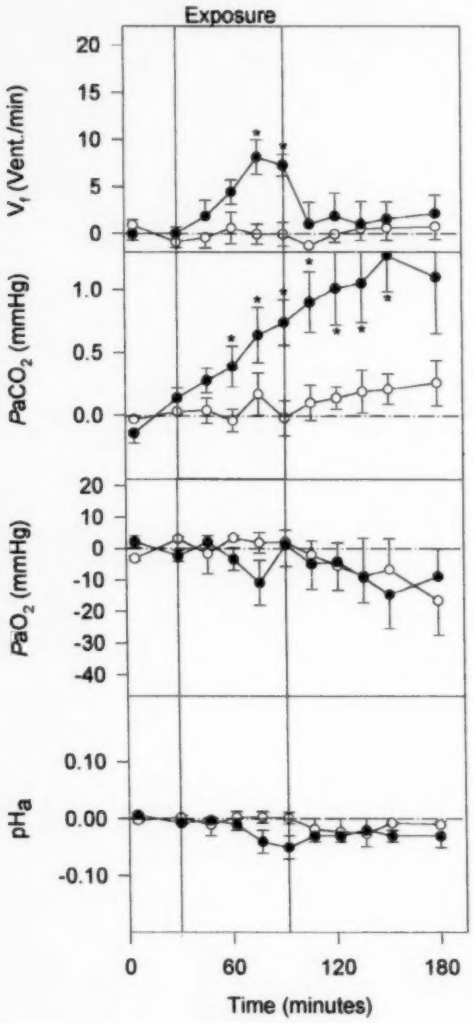
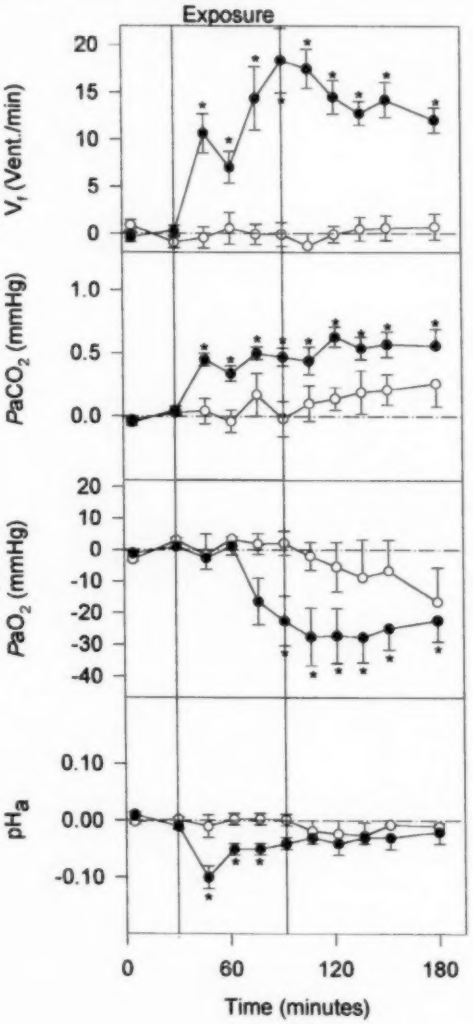


Fig. 3. The change in ventilation frequency (V_f), arterial P_{CO_2} (Pa_{CO_2}), arterial P_{O_2} (Pa_{O_2}), and arterial pH (pH_a) during exposure to 0.45 mg·L^{-1} sodium hypochlorite (a.i.) (●). The chemical exposure period is represented between the two vertical bars. ○, control fish; *, significantly different from base-line zero. Error bars represent 1 SE.



significant effect of treatment on any blood parameter (Table 1). Despite significant decreases in haematocrit and haemoglobin concentration after 1-h postexposure to 2 mg·L^{-1} chloramine T, there was no significant effect of exposure (to any of the chemicals in this study) on the O_2 specifically bound to haemoglobin (O_2–Hb) or mean cellular haemoglobin concentration (Table 1). Similarly there was no significant increase in plasma catecholamine levels in any of the treatments (Table 2).

Acid–base status

It was evident from the pH–bicarbonate diagrams that exposure to chloramine T resulted in an apparently mixed respiratory and metabolic acidosis at 9 mg·L^{-1} chloramine T. However, the point representing 60 min post-exposure was close to the buffer line (Fig. 5A). At 2 mg·L^{-1} chloramine T there was a respiratory acidosis superimposed over a metabolic alkalosis (Fig. 5B). However, fish exposed to 0.45 mg·L^{-1} sodium hypochlorite exhibited a purely respiratory acidosis (Fig. 5C), whereas fish exposed to 9 mg·L^{-1} *p*TSA exhibited a purely metabolic acidosis (Fig. 5D).

Discussion

A striking feature of exposure to chloramine T and hypochlorite is the significant increase in V_f. However, despite this

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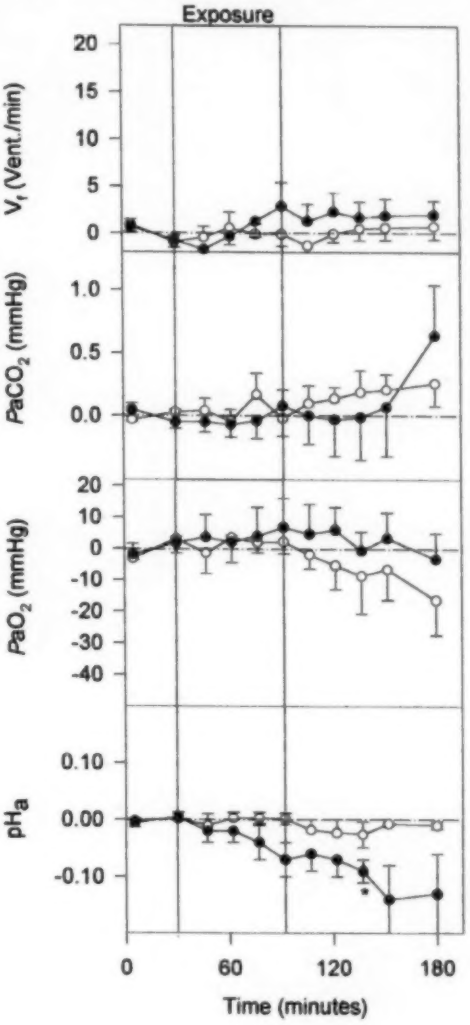
apparent hyperventilation, P_{aCO_2} was significantly elevated over base-line values. Generally, hyperventilation is associated with a lowering of P_{aCO_2} (Iwama et al. 1987). As a consequence of this it would be predicted that P_{aCO_2} should have decreased during the periods of increased ventilation. However, this did not occur. The fact that in this experiment P_{aCO_2} increased despite gill hyperventilation suggests a change in the diffusive conductance of the gill that is impairing CO_2 excretion. A possible explanation for this is the secretion of mucus onto the gill surface, thereby increasing the blood to water diffusion distance and (or) unstirred boundary layer of the gill. This would, on the one hand, cause a rise in P_{aCO_2} because the gill is believed to be diffusion limited with respect to CO_2 transfer (Cameron and Polhemus 1974; Malte and Weber 1985). On the other hand, the trout gill is principally perfusion limited with respect to O_2 transfer (Daxboeck et al. 1982; Randall and Daxboeck 1984; Malte and Weber 1985). Thus, under normoxic conditions, branchial oxygen uptake would not likely be limited by the thickness of the diffusion barrier, and hence P_{aO_2} was unaffected by chloramine T (and presumably the mucous release caused by chloramine T). This notwithstanding, Bass and Heath (1977), using a similar concentration of sodium hypochlorite to that used in the present study, noted a lowering of P_{aO_2} associated with hypersecretion of mucus on the gill of repeatedly exposed rainbow trout (Bass et al. 1977). The argument above, however, does not hold for hypochlorite because Bass et al. (1977) also noted morphological changes in the gill, leading to a reduction in the gill surface area as well as diffusion distance. Nevertheless, hypochlorite exposure caused an elevation in P_{aCO_2} in this study, and since this relationship between V_f and P_{aCO_2} was not observed with *p*TSA exposure, it strongly suggests that this hyperventilatory response and the elevation in P_{aCO_2} are caused by the liberation of hypochlorite from chloramine T. The apparent impairment of CO_2 excretion cannot be explained by chloramine T induced inhibition of red blood cell HCO_3^- dehydration because there was no effect of chloramine T on the rate of red blood cell CO_2 excretion (M.D. Powell and S.F. Perry, unpublished results). Using the radioisotopic HCO_3^- dehydration assay of Wood and Perry (1991), red blood cell carbonic anhydrase activity was unaltered, and this infers that inhibition of boundary-layer carbonic anhydrase (Wright et al. 1989) was also unaltered by chloramine T and therefore not a factor in the elevation of P_{aCO_2}.

Upon the withdrawal of chloramine T and subsequent recovery of the fish, ventilation frequency decreased to base-line levels either within the first 15 min (2 mg·L^{-1}) or over the subsequent 90-min recovery period (9 mg·L^{-1}). Over this recovery period, P_{aCO_2} levels in the fish treated with 2 mg·L^{-1} remained elevated and even continued to rise slightly, whereas in the fish treated with 9 mg·L^{-1}, P_{aCO_2} levels gradually returned to base-line levels within 90 min. The P_{aCO_2} of fish treated with hypochlorite showed a persistent elevation despite the significantly elevated V_f. This further suggests that elevation in P_{aCO_2} is caused by reduced gill conductance, perhaps owing to mucus secretion. When hyperventilation persisted even after the withdrawal of chloramine T (Fig. 1) and the stimulus for mucus secretion was removed, the P_{CO_2} levels were reduced to base-line levels. With the withdrawal of 2 mg·L^{-1} chloramine T, hyperventilation ceased almost immediately, and as the maintenance of hyperventilation that would

Fig. 4. The change in ventilation frequency (V_f), arterial P_{CO_2} (P_{aCO_2}), arterial P_{O_2} (P_{aO_2}), and arterial pH (pH_a) during exposure to 9 mg·L^{-1} paratoluenesulphonamide (*p*TSA) (a.i.) (●). The chemical exposure period is represented between the two vertical bars. ○, control fish; *, significantly different from base-line zero. Error bars represent 1 SE.



lead to excretion of this excess CO_2 was not present maintained, therefore CO_2 continued to build up in the blood. With hypochlorite, the hyperventilation was sufficient to maintain P_{aCO_2}, albeit at an elevated level. This hyperventilation may have been maintained as a consequence of the significant lowering of P_{aO_2} by 30 mmHg (1 mmHg = 133.3 Pa), from approximately 97 to 68 mmHg (Fig. 3). This result is consistent with the findings of Bass and Heath (1977), in which after a single 3-h pulse of a peak concentration (0.4 mg·L^{-1}), total residual chlorine P_{aO_2} was reduced by approximately 60 mmHg, compared with 30 mmHg after a 1-h pulse in our study. However, in the present study, the lowering of P_{aO_2} was

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Table 1. Mean (± SEM) oxygen content (Cao_2, mL O_2/100 mL blood), haematocrit (%), haemoglobin (Hb) concentration (g Hb/100 mL blood), O_2 specifically bound to haemoglobin (O_2–Hb, mL O_2/g Hb), and mean cellular haemoglobin concentration (MCHC, g·mL^{-1}) of rainbow trout arterial blood before and after exposure to chloramine T (CLT–1 at 2 mg·L^{-1} and CLT–2 at 9 mg·L^{-1}), paratoluenesulphonamide (*p*TSA at 9 mg·L^{-1}), or sodium hypochlorite (NaOCl at 0.45 mg·L^{-1}).

Treatment	Pre-exposure	60-min exposure	1 h post-exposure
Cao_2			
Control	6.83±0.69	5.86±0.65	5.08±0.72
CLT-1	8.97±0.90	8.47±0.97	7.06±0.74
CLT-2	10.70±0.99	9.34±0.93*	7.89±0.53*
NaOCl	7.60±1.21	7.73±0.97	6.91±0.87
*p*TSA	9.53±1.21	9.90±1.01*	8.96±1.24
Haematocrit			
Control	22.0±2.4	19.2±2.4	16.1±1.6
CLT-1	22.2±0.9	20.5±1.3	18.1±0.8**
CLT-2	27.3±2.4	25.1±2.1	23.0±1.7
NaOCl	19.7±2.4	20.8±2.3	19.6±1.9
*p*TSA	25.5±1.7	24.5±1.7	23.7±2.0
Haemoglobin			
Control	6.74±0.77	5.76±0.59	5.19±0.60*
CLT-1	8.46±0.46	7.81±0.42	6.47±3.75**
CLT-2	9.10±0.77	8.79±0.67*	7.31±0.52
NaOCl	6.80±0.92	7.35±0.92	6.35±0.70
*p*TSA	9.77±0.86	9.24±0.71*	8.10±0.73*
O_2–Hb			
Control	0.95±0.06	0.92±0.03	0.86±0.05
CLT-1	0.99±0.08	1.01±0.09	1.01±0.09
CLT-2	1.13±0.08	1.02±0.09	1.01±0.04
NaOCl	1.01±0.10	0.98±0.03	0.98±0.08
*p*TSA	0.92±0.10	1.01±0.07	1.01±0.07
MCHC			
Control	0.31±.01	0.30±0.01	0.32±0.01
CLT-1	0.38±0.02*	0.38±0.02*	0.36±0.01
CLT-2	0.33±0.01	0.35±0.02	0.32±0.01
NaOCl	0.34±0.01	0.35±0.01*	0.33±0.01
*p*TSA	0.38±0.01*	0.38±0.01*	0.34±0.01

*Significantly different from control ($p < 0.05$).
**Significantly different from pre-exposure ($p < 0.05$).

insufficient to cause significantly depress blood O_2 content. Thus, the hyperventilation that occurred during exposure to chloramine T (no change in blood Po_2 or content) or hypochlorite (change in Pa_{O2} only) was unlikely to have been induced by hypoxemia (Smith and Jones 1982; see review by Shelton et al. 1986). Similarly, plasma catecholamine levels were not substantially elevated (see below) and could not have contributed to the hyperventilatory response (Randall and Taylor 1991). It is possible that the stimulus for ventilation originated in the central nervous system, perhaps through stimulation of nociceptors by hypochlorite or chloramine T.

There were no significant effects of treatment on total oxygen content, haematocrit, or haemoglobin content. The decreases in haematocrit and haemoglobin were probably due to the removal of blood within a short period of time (1.95 mL within 3 h) and therefore likely were artefacts of repeated sampling. Similarly, there was no consistent effect of treatment on

Table 2. Mean (± SEM) plasma catecholamine concentrations (nmol·L^{-1}) in rainbow trout arterial blood before and after exposure to chloramine T (CLT–1 at 2 mg·L^{-1} and CLT–2 at 9 mg·L^{-1}), sodium hypochlorite (NaOCl at 0.45 mg·L^{-1}), or paratoluenesulphonamide (*p*TSA at 9 mg·L^{-1}).

Treatment	Pre-exposure	60-min exposure	1 h post-exposure
Adrenaline			
Control	4.88±1.94	6.10±4.88	3.08±0.98
CLT-1	0.60±0.38	3.67±1.60	2.54±1.04
CLT-2	4.27±1.53	12.53±3.16	11.70±4.75
NaOCl	2.85±1.46	13.08±6.90	27.80±19.1
*p*TSA	5.20±4.63	6.14±3.23	16.19±4.41
Noradrenaline			
Control	2.96±1.52	1.71±1.14	3.69±1.42
CLT-2	4.90±1.41	4.46±1.44	7.25±2.14
NaOCl	1.28±1.28	8.94±5.26	7.49±2.58
*p*TSA	2.54±1.52	5.28±1.75	6.81±3.01

catecholamine concentrations. Although there were elevations of catecholamines in some individual fish (as represented by a variance) (Table 2), the levels were considerably lower than those measured in the recovery of stressed fish (Gamperl et al. 1994) and probably of little physiological significance. Indeed, the levels of adrenaline and noradrenaline during exposure were substantially below those levels required to elicit red cell adrenergic responses (Tetens et al. 1988). This is reflected by the absence of any decrease in the mean cellular haemoglobin concentration that would be expected had there been any cell swelling owing to an influx of water following β-adrenergic activation of the Na^+–H^+ exchange (Nikinmaa 1992).

In the extracorporeal circulation of trout, the arterial blood is in a state of acid–base disequilibrium (Gilmour et al. 1994) and therefore some small error may be associated with using values of $Paco_2$ and pH_a in equilibrium calculations. Nevertheless, since there was no significant acidosis with chloramine T or elevation in catecholamine levels that serve to enhance the disequilibrium (Gilmour and Perry 1995), these calculations remain valid.

Chloramine T and perhaps hypochlorite appear to affect the acid–base status of trout, but the exact nature of these effects is not clear. It was evident from this study that exposure to 9 mg·L^{-1} chloramine T caused an acidosis, primarily respiratory in nature with a small metabolic component. With the withdrawal of chloramine T, the acidosis was entirely respiratory (Fig. 5A). Hypochlorite caused an entirely respiratory acidosis and *p*TSA a metabolic acidosis. Perhaps the effects shown by chloramine T at 9 mg·L^{-1} represent the combined effects of these chemicals. However, exposure to 2 mg·L^{-1} chloramine T resulted in a respiratory acidosis and a pronounced metabolic alkalosis with the withdrawal of chloramine T (Fig. 5B). At present we can only speculate as to the causes of such disturbances. The development of a respiratory acidosis is consistent with our contention that chloramine T and hypochlorite caused a hypersecretion of branchial mucus that was sufficient to impair CO_2 excretion. However, it would appear that there are metabolic components to the acid–base disturbances that probably reflect changes in branchial acid or base fluxes. We cannot discount the possibility that in fish treated with 9 mg·L^{-1} chloramine T or hypochlorite, metabolic

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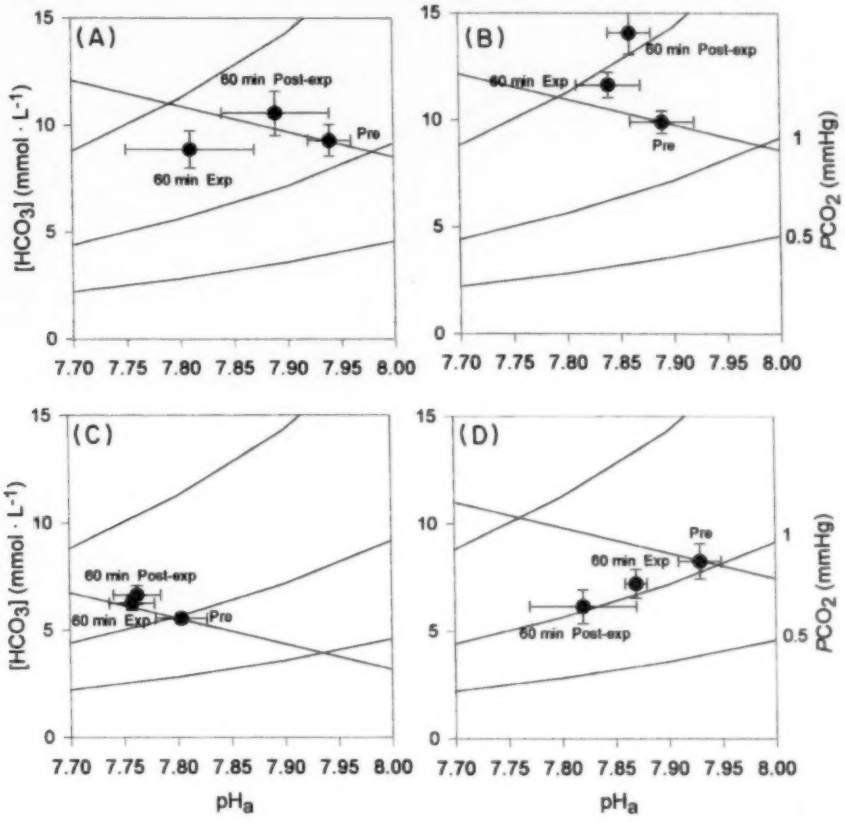
Fig. 5. The relationship between plasma bicarbonate (HCO_3^-) and arterial pH (pH_a) for the blood of trout treated (●) with (A) 9 mg·L^{-1} chloramine T, (B) 2 mg·L^{-1} chloramine T, (C) 0.45 mg·L^{-1} sodium hypochlorite, and (D) 9 mg·L^{-1} *p*TSA. Error bars represent 1 SE.



compensation and a subsequent metabolic alkalosis were not complete by the end of the experimental period.

The acid–base disturbances seen in this study correlate well with the changes in chloride cell ultrastructure seen from rainbow trout that had been intermittently exposed to chloramine T at similar concentrations (Powell et al. 1995). Gill chloride cells from intermittently exposed fish expressed a significantly greater proportion of the apical plasmalemma than control fish. Such a morphological response is similar to that seen in fish in which a metabolic alkalosis had been induced (Goss et al. 1992*a*, 1992*b*; Goss and Perry 1993).

With the exception of acid–base disturbances and since there is no significant impairment of O_2 uptake nor any significant release of catecholamines, we conclude that a single chloramine T exposure of healthy rainbow trout at therapeutic concentrations used in the aquaculture industry appears to have little pathophysiological impact.

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Comparative demography of two populations of the bonnethead shark (*Sphyrna tiburo*)

Enric Cortés and Glenn R. Parsons

Abstract: Demographic analyses were conducted for two populations of the bonnethead shark (*Sphyrna tiburo*) inhabiting two bays of southwest Florida. Survivorship values for the bonnethead shark were estimated through two theoretical relationships for Florida Bay and additionally through two length-converted methods for Tampa Bay. Net reproductive rates per generation ranged from 1.04 to 3.45, generation lengths from 4.0 to 5.1 years, and instantaneous rates of population increase from 0.011 to 0.272 for the Tampa Bay population. For the Florida Bay population, net reproductive rates per generation ranged from 1.32 to 3.61, generation lengths from 4.1 to 5.1 years, and instantaneous rates of population increase from 0.068 to 0.283. Predicted stable age distribution, mean expectation of further life, and reproductive values for females from the two populations were very similar. Although the life tables constructed indicated slightly faster population growth in Florida Bay, we believe this is a result of the methods used to estimate mortality. Environmental factors or heritability may account for the life history differences between these two populations. In all, instantaneous rates of population increase for this species are considerably higher than those computed for other species of elasmobranchs using similar methodology.

Résumé : On a effectué des analyses démographiques sur deux populations de requins marteau tiburo (*Sphyrna tiburo*) vivant dans deux baies du sud-ouest de la Floride. On a évalué les taux de survie du requin marteau tiburo à l'aide de deux relations théoriques pour la baie Florida, ainsi qu'à l'aide de deux méthodes converties pour tenir compte de la taille, pour la baie Tampa. Les taux de reproduction nets par génération variaient de 1,04 à 3,45, la durée de la génération allait de 4,0 à 5,1 ans et les taux instantanés d'accroissement de la population variaient de 0,011 à 0,272 pour la population de la baie Tampa. Dans le cas de la population de la baie Florida, les taux de reproduction nets par génération variait de 1,32 à 3,61, la durée de la génération allait de 4,1 à 5,1 ans, tandis que les taux instantanés d'accroissement de la population variait de 0,068 à 0,283. La composition par âge stable prévue, l'espérance moyenne de vivre plus longtemps et la valeur de reproduction pour des femelles des deux populations étaient semblables. Les tables de survie que nous avons élaborées indiquaient un accroissement de population légèrement plus élevé dans la baie Florida; néanmoins, cette différence est le résultat, estimons-nous, des méthodes utilisées pour évaluer la mortalité. Les facteurs environnementaux ou l'héritabilité peuvent expliquer les différences d'évolution biologique chez ces deux populations. En somme, les taux instantanés d'accroissement de la population pour ces espèces sont considérablement plus élevées que celles calculées pour d'autres espèces d'élasmobranches à l'aide d'une méthodologie semblable.
[Traduit par la Rédaction]

Introduction

Population dynamics and demography of elasmobranch fishes are still poorly understood. The paucity of knowledge is due to the difficulty in studying elasmobranch populations and assessing their sizes and to the lack of basic biological and ecological information for numerous species, which is in turn due to the relatively low production and economic value of elasmobranchs compared with teleosts. Thus, from the approximately 400 extant species of sharks, demographic parameters or other aspects of population dynamics have only been estimated for 8 species: the spiny dogfish (*Squalus acanthias*, Jones and Geen 1977; Wood et al. 1979), the lemon shark (*Negaprion brevirostris*, Hoenig and Gruber 1990), the sandbar shark (*Carcharhinus plumbeus*, Hoff 1990), the leopard shark (*Triakis semifasciata*, Smith and Abramson 1990; Cailliet 1992), the Pacific angel shark (*Squatina californica*, Cailliet et al. 1992), the Atlantic sharpnose shark (*Rhizoprionodon terraenovae*, Cortés 1995), the Australian school shark (*Galeorhinus australis*, Grant et al. 1979), and the gummy shark (*Mustelus antarcticus*, Walker 1992, 1994*a*, 1994*b*).

Sharks and the other elasmobranch fishes (skates and rays) possess life history traits that differ markedly from those of most teleosts, approaching those of K-selected species such as marine and some terrestrial mammals. They tend to have slow growth, are long lived, and have late sexual maturity, long gestation periods, and few offspring and, unlike teleosts, parental stock and recruitment are closely related, making them vulnerable to exploitation (Holden 1974, 1977). Despite the increasing interest in several elasmobranch species because of their importance to fisheries, sufficient information to understand the population biology of most exploited and unexploited populations is not available (Compagno 1990).

The bonnethead shark (*Sphyrna tiburo*) is an abundant small coastal species commonly found in shallow-water environments of the U.S. Atlantic, Gulf of Mexico, and Central and

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E. Cortés.[1] Center for Shark Research, Mote Marine Laboratory, Sarasota, FL 34236, U.S.A.
G.R. Parsons. Department of Biology, University of Mississippi, MS 38677, U.S.A.

[1] Author to whom correspondence should be addressed.
e-mail: ecortes@marinelab.sarasota.fl.us

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South American Atlantic coasts (Compagno 1984). While bonnethead sharks are harvested in coastal waters of Trinidad and Tobago (Henry 1992) and the southern Gulf of Mexico (Bonfil et al. 1990), there are no directed fisheries for this species or significant by-catch from other fisheries in the eastern Gulf of Mexico. Ongoing tagging studies along the west coast of Florida have so far failed to reveal any long-distance migrations in this species, suggesting that bonnethead sharks are not highly migratory and that fisheries in other areas probably do not affect the Florida stocks.

Several life history aspects of this species have been well studied, including its food and feeding habits (Cortés et al. 1996), age and growth (Parsons 1993*a*), and reproductive biology (Parsons 1993*b*). More importantly, the annual periodicity of growth zones in the vertebrae was validated by maintaining individuals injected with oxytetracycline in captivity (Parsons 1993*a*). Reliable age estimates can thus be used in conjunction with available reproductive information to construct life tables. However, the absence of mortality estimates for this species has prevented demographical modelling of any of its populations.

The objectives of this study are threefold. First, we attempted to estimate mortality for two populations of the bonnethead shark from southwest Florida using four methods. Second, we developed life tables for these two populations under several mortality–survivorship scenarios and compared them in the light of known life history traits. Third, we wished to compare life history strategies and demographic parameters for the bonnethead shark with those derived for other species of sharks. This study represents the first attempt to estimate mortality in a species of shark through a variety of methods and to compare life tables for two populations of a single species of shark off the U.S.A.

Materials and methods

Study sites

Both Tampa and Florida bays are shallow estuaries situated in southwest Florida. Tampa Bay is a relatively turbid body of coastal water, close to human settlements; Florida Bay is a more open body of water situated in the Florida Keys and overall appears to be a more pristine environment (Parsons 1993*b*). Detailed descriptions of both sites are available in the literature (see Lewis and Estevez 1988 for Tampa Bay; Schmidt 1979 and Schomer and Drew 1982 for Florida Bay). The mean annual water temperature in Florida Bay measured from 1984 to 1985 was 25.8°C (Holmquist et al. 1989), while that in Tampa Bay, based on four decades of records, was 22.3°C (Lewis and Estevez 1988).

Vertebral samples used to calculate validated age and growth and reproductive information previously published (Parsons 1993*a*, 1993*b*) came from bonnethead sharks collected in Tampa Bay (27°45′N, 82°40′W; N = 48 males, 96 females) and Florida Bay (24°45′N, 81°00′W; N = 44 males, 45 females) from July 1982 to December 1986. Additionally, bonnethead sharks collected in Tampa Bay from December 1992 to November 1993 (N = 202 males, 402 females) as part of a nursery ground study (Hueter and Manire 1994) were used to estimate mortality through length-converted methods: these specimens were collected over grass flats using 11.4-cm stretched mesh gill nets set for 0.8–1.5 h in depths ranging from 0.3 to 7 m. All sharks caught were measured (precaudal, fork, and total lengths (TL) to the nearest centimetre) and weighed (to the nearest 0.1 kg).

Life tables

We constructed separate life tables for the Tampa Bay and the Florida Bay populations on the basis of our best estimates of longevity, age at maturity, fecundity, and mortality, assuming that the populations were isolated, i.e., no emigration or immigration took place. From these, we calculated estimates of net reproductive rate per generation (R_0), mean generation time (G, yr), intrinsic rate of population increase (r, yr^{-1}) corrected through the Euler equation, and population doubling time ($t_{\times 2} = \ln 2/r$, yr) using standard demographic techniques (Wilson and Bossert 1971; Caughley 1977; Krebs 1985). In addition, we also calculated the stable age distribution (C_x), the mean expectation of further life for females at age (e_x, yr), and the reproductive value for females at age (V_x, number of female offspring that remain to be born to each female of age x).

Age at maturity, maximum age, and fecundity

Ages at maturity for female bonnethead sharks from Tampa Bay and Florida Bay were estimated to vary from ≈2.0 to 2.4 yr and from ≈2.4 to 3.0 yr (Parsons 1993*a*), respectively. Because parturition in the bonnethead shark occurs annually around late August or early September after a 4.5- to 5-month gestation period (Parsons 1993*b*), any age 2 female shark maturing before the summer would not produce any pups until August or September. Thus, for purposes of the demographic analyses, we assumed that females would not be reproductively active until their third birthday in both bays.

Parsons (1993*a*) estimated that female bonnethead sharks from both sites reach their theoretical maximum size at age 12+. We chose to use a conservative estimate of life-span of 12 yr on the basis of a preliminary sensitivity analysis that revealed that increasing longevity by up to 3 yr (to 15 yr) only very slightly affected the estimate of population rate of increase (<1%).

Several aspects of fecundity were considered to estimate the age-specific natality function (m_x): mean litter size (the average number of embryos in a litter), periodicity of parturition, percentage of mature females at age, percentage of mature females reproductively active in any given year, and sex ratio at birth.

Because no significant correlation was found between the total length of gravid females and the number of offspring produced by this species in either of the two sites, we used a mean litter size of 8.9 and 9.3 pups/yr in Tampa Bay and Florida Bay, respectively (Parsons 1993*b*). Periodicity of births for females of both populations is annual and sex ratios at birth are very close to 1:1 (Parsons 1993*b*), so we divided the values of mean litter size by 2 to obtain mean annual fecundity values of 4.45 and 4.65 female pups/female in Tampa Bay and Florida Bay, respectively. No further adjustments were done to these figures because we assumed that all females older than the age at maturity are indeed mature and that all mature females breed every year.

Mortality and survivorship

We used four methods to estimate mortality and survivorship. Two of these methods rely on theoretical relationships between mortality and parameters from the von Bertalanffy growth function (VBGF); the other two are length-converted methods that require a large sample size and could only be applied to the Tampa Bay population of bonnethead sharks.

(*i*) Hoenig's (1983) equation relates maximum age to the total instantaneous mortality rate (Z) and was derived from data pertaining to 84 mostly unexploited or lightly exploited fish stocks:

$$\ln(Z) = 1.46 - 1.01 \ln(t_{max})$$

where t_{max} (12 yr) is longevity in years. Because there is no fishery directed at this species or significant by-catch from other fisheries in the study areas, Z can be approximated to M (natural instantaneous mortality rate).

(*ii*) Pauly's (1980) equation relates M to VBGF parameters and

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an estimate of the mean annual temperature of the water in which the population studied occurs:

$$\log M = -0.0066 - 0.279\log L_\infty + 0.6543\log K + 0.4634\log \bar{T}$$

where L_∞ is the asymptotic total length from the VBGF, in centimetres; K is the Brody growth coefficient from the VBGF; and $\bar{T}$ is mean annual water temperature. We used the following input parameters for this equation: for Tampa Bay, $L_\infty = 115$ cm and $K = 0.34$ for females, $\bar{T} = 22.3$°C; for Florida Bay, $L_\infty = 103.3$ cm and $K = 0.37$ for females, $\bar{T} = 25.8$°C (Parsons 1993*a*).

(*iii*) A length-converted catch curve (LCCC–1) (Baranov 1918; Pauly 1984) was calculated from the VBGF using 402 female bonnethead sharks collected in Tampa Bay. Each individual length was converted to an integer age, and the age groups (x axis) were plotted against the natural logarithm of the number of individuals in each age group (y axis). The slope (b) of the descending right limb of the catch curve is found through least-squares linear regression, with b estimating –Z.

(*iv*) Finally, a deterministic age–length key (Hilborn and Walters 1992) was used to calculate Z for the sample of 402 female bonnethead sharks from Tampa Bay, through a length-converted catch curve (LCCC-2) similar to the one just described (Pauly 1984; Hoenig et al. 1987). Lengths were grouped in intervals and a relative age corresponding to the midpoint of each length interval was calculated as $t' = -\ln(1 - L/L_\infty)$ and plotted against the natural logarithm of the number of individuals in each length interval. The slope from the descending right limb of this length-converted catch curve estimates $1 - (Z/K)$, and thus Z can be obtained. The first point to be included in the linear regression is that immediately to the right of the highest point and any points obtained through conversion of lengths within 5% of L_∞ are not included in the calculation (Pauly 1984). Additionally, one single outlier may be excluded from the calculation of Z (Pauly 1984).

Both length-converted catch curves implicitly assume a constant Z, constant recruitment, and no gear selectivity in all age groups used in the calculation.

The proportion of survivors at the start of each age interval (x) was obtained as

$$l_x = \prod_{y=0}^{x-1} S_y$$

i.e., l_x was calculated as the product of all of the S_y values from S_0 to S_{x-1}, where S_y is survival from age y to age $y + 1$, with $S = e^{-Z}$.

We used two levels of survivorship to calculate the life tables. On the basis of the results of a tagging and removal experiment conducted by Manire and Gruber (1993) on lemon sharks, we assumed that survivorship during the 1st year of life was 47.5% ($S_0 = 0.475$) by taking the midpoint of their calculated and minimum estimates of annual mortality rate and converting it to a survivorship value. Survivorship for age 1+ sharks was assumed to be constant (for age groups 1–11).

Results

Survivorship

Survivorship for age 1+ bonnethead sharks from Tampa Bay varied from 0.498 to 0.705 according to the method used. For age 1+ bonnethead sharks from Florida Bay, the two methods yielded values of 0.529 and 0.705 (Table 1).

We considered the value of $Z = 0.697$ obtained through LCCC-1 to be a valid estimate for Tampa Bay, because the linear regression of age against the natural logarithm of the number of individuals in each age group was significant ($P < 0.01$, $r^2 = 0.96$, $N = 5$) and the residuals were normally distributed ($P > 0.05$) and had constant variance ($P > 0.05$). Likewise, we chose a 2-cm length interval to calculate a value of $Z = 0.626$ through LCCC-2 because it gave the best fit to the linear regression of relative age against the natural logarithm of the number of individuals in each length interval ($P < 0.01$, $r^2 = 0.86$, $N = 7$) and the regression assumptions of normality and homoscedacity were also met ($P > 0.05$). Annual survivorship for female age 1+ bonnethead sharks from Tampa Bay is thus $e^{-0.697} = 0.498$ (approximate 95% confidence limits: 0.393–0.631) according to LCCC-1 (Table 1) and $e^{-0.626} = 0.535$ (approximate 95% confidence limits: 0.471–0.638) according to LCCC-2 (Fig. 1; Table 1). We could not calculate a value of Z for bonnethead sharks from Florida Bay with either of these two methods because our sample size was too small ($N = 54$) and the regression assumptions were not met.

Table 1. Estimates of mortality and survivorship for age 1+ female bonnethead sharks from Tampa and Florida bays, southwest Florida, derived using four methods.

Computed statistic	Method			
	Hoenig's	Pauly's	LCCC-1	LCCC-2
Tampa Bay				
b	—	—	–0.697	–0.842
Z	0.350	0.545	0.697	0.626
95% CL (Z)	—	—	0.461–0.933	0.470–1.214
S	0.705	0.580	0.498	0.535
95% CL (S)	—	—	0.393–0.631	0.471–0.638
r^2	—	—	0.96	0.86
Florida Bay				
Z	0.350	0.635	—	—
S	0.705	0.529	—	—

Note: Only two methods could be used for the Florida Bay population because of small sample size.

Life tables

On the basis of the survivorship results described above, we present four alternative life tables for the Tampa Bay population corresponding to the four methods used to calculate mortality for age 1+ sharks (Table 2) and two life tables for the Florida Bay population (Table 3) to provide some insight into the variability that may be operating on the two populations. The Tampa Bay population of bonnethead sharks could be increasing at a rate between 1%/yr ($e^r = e^{0.011} = 1.011$) in the worst case scenario (scenario D) and 31%/yr ($e^r = e^{0.272} = 1.312$) in the best case scenario (scenario A; Table 2). In Florida Bay, the rate of population increase is between 7%/yr ($e^r = e^{0.068} = 1.070$) and 33%/yr ($e^r = e^{0.283} = 1.327$; Table 3).

In Tampa Bay, mean generation time (G) was ≈5.1 yr when the highest value of survivorship for age 1+ sharks was used (scenario A) and decreased to ≈4 yr for the lowest survivorship value (scenario D; Table 2). In Florida Bay, G decreased from ≈5.1 yr when using the largest value of survivorship (scenario A) to ≈4.1 yr when using the lowest value of survivorship (scenario B; Table 3). In general, G, r, and the multiplication rate per generation (R_0) decreased whereas population doubling time ($t_{\times 2}$) increased with decreasing values of survivorship for age 1+ sharks (Tables 2 and 3).

The stable age distributions (C_x) calculated for bonnethead sharks under all of the scenarios in both bays were almost

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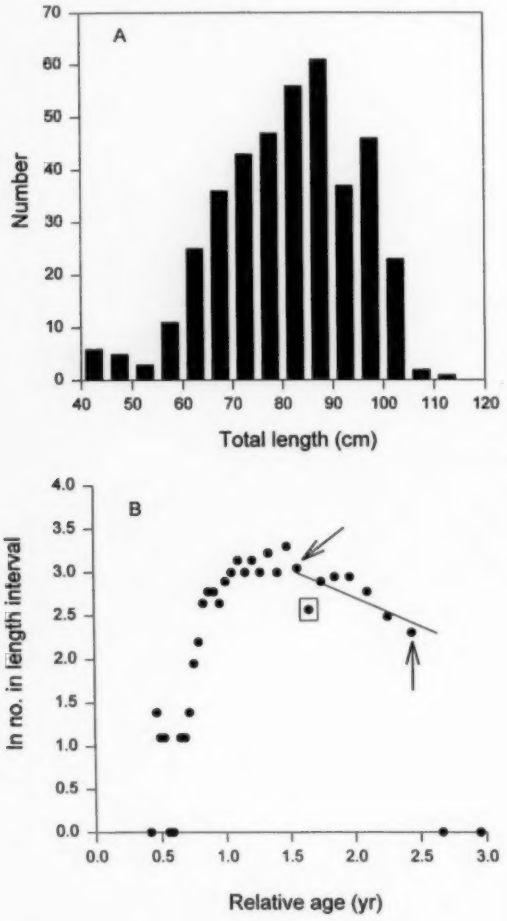
Fig. 1. (A) Length-frequency distribution of 402 female bonnethead sharks from Tampa Bay and (B) length-converted catch curve (LCCC-2) derived from data above. The ordinate is the natural logarithm of the number of sharks in a 2-cm length interval; the abscissa is the relative age at the midpoint of the 2-cm length interval. The arrows indicate the first and last points used to calculate the regression; the point inside the box is an outlier excluded from the calculation.



identical and were dominated by immature individuals (Fig. 2). Thus, age 0 sharks constituted more than 50% of the population, age 1 sharks more than 20%, and age 2 sharks more than 10%, in all cases.

The largest values of mean expectation of further life (e_x) for all scenarios in both bays corresponded to age 1 bonnethead sharks (Fig. 3). Thus, age 1 sharks can be expected to live from approximately 2.8 more years in both bays (scenario A) to 1.5 more years (scenario D; Tampa Bay). After the 1st year of life, expectation of further life progressively decreases as sharks approach their maximum age (Fig. 3).

Reproductive value at each age (V_x) rapidly rises in all scenarios to a peak for age 3 sharks and then falls gradually (Fig. 4). Just after reaching maturity, an age 3 female shark in Tampa Bay can expect to have between 8 and 10 female offspring, while an age 3 female from Florida Bay can expect to have 9 or 10 female offspring before it dies. The somewhat higher values for bonnethead sharks from Florida Bay given equal survivorship (scenario A for both populations and scenario C for Tampa Bay versus scenario B for Florida Bay) arise from the higher fecundity of females in that area despite their later maturity (2.7 yr in Florida Bay versus 2.2 yr in Tampa Bay).

Discussion

Fecundity and longevity

Parsons (1993*b*) reported marked differences in reproductive parameters of bonnethead sharks from Tampa and Florida bays. Individuals from the Tampa Bay population produce fewer but larger offspring, and in general invest a larger proportion of their energy budget in reproduction. Mean embryo length and mass at birth for female bonnethead sharks in Tampa Bay were 35 cm and 172 g compared with 27 cm and 82 g for individuals in Florida Bay (Parsons 1993*b*). Survivorship of young individuals, especially newborn pups, may be affected by size-selective predation. Thus, the smaller, slower growing age 0 individuals from the Florida Bay population are likely to be more vulnerable to predators than their larger, faster growing counterparts in Tampa Bay, resulting in decreased survivorship for the former. Decreased survivorship of age 0 individuals in Florida Bay with respect to those in Tampa Bay was not contemplated in our life tables but would have resulted in smaller intrinsic rates of increase for the Florida Bay population.

Parsons (1993*b*) also reported a higher rate of infertility in female bonnethead sharks from Tampa Bay (27%) than in females from Florida Bay (4.3%). He noted that neither precocity nor senescence accounted for these observations because no correlation could be found between the size of the shark and the incidence of infertility. Since population regulation through infertility appears to be very energetically inefficient and ecologically unsound, in the absence of any other evidence, environmental quality may be the causative agent. The higher rate of infertility for the Tampa Bay population may be explained by the fact that Tampa Bay is a less pristine environment than Florida Bay. Although these rates of infertility were derived for sharks collected from 1982 to 1986, recent findings indicate that they are still high, at least in the Tampa Bay population (16.7%; C.A. Manire and R.E. Hueter, Center for Shark Research, Mote Marine Laboratory, Sarasota, FL 34236, U.S.A., personal communication). Calculation of population growth rates assuming that infertile ova had all been fertile indicates that for scenario A, r would have increased by 22% (from 0.272 to 0.331) in Tampa Bay and by only 3.5% (from 0.283 to 0.293) in Florida Bay. If environmental degradation is indeed the cause of the high infertility rate in Tampa Bay, population growth in that area is being greatly affected.

Our estimate of longevity for female sharks from Tampa and Florida bays (12 yr in both cases) used in the life tables was based on Parsons (1993*a*): while no sharks older than 6+ yr were actually aged, predictions from his VBGF indicated

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Table 2. Survivorship and fertility tables for bonnethead sharks from Tampa Bay, representing four scenarios based on four different values of survivorship for sharks aged 1+ yr (S) and constant 1st yr survivorship ($S_0 = 0.475$).

	Scenario A			Scenario B			Scenario C			Scenario D		
	l_x	m_x	l_xm_x	l_x	m_x	l_xm_x	l_x	m_x	l_xm_x	l_x	m_x	l_xm_x
0	1.000	0	0	1.000	0	0	1.000	0	0	1.000	0	0
1	0.475	0	0	0.475	0	0	0.475	0	0	0.475	0	0
2	0.335	0	0	0.276	0	0	0.254	0	0	0.237	0	0
3	0.236	4.45	1.051	0.160	4.45	0.711	0.136	4.45	0.605	0.118	4.45	0.524
4	0.166	4.45	0.741	0.093	4.45	0.412	0.073	4.45	0.324	0.059	4.45	0.261
5	0.117	4.45	0.522	0.054	4.45	0.239	0.039	4.45	0.173	0.029	4.45	0.130
6	0.083	4.45	0.368	0.031	4.45	0.139	0.021	4.45	0.093	0.015	4.45	0.065
7	0.058	4.45	0.260	0.018	4.45	0.080	0.011	4.45	0.050	0.007	4.45	0.032
8	0.041	4.45	0.183	0.010	4.45	0.047	0.006	4.45	0.027	0.004	4.45	0.016
9	0.029	4.45	0.129	0.006	4.45	0.027	0.003	4.45	0.014	0.002	4.45	0.008
10	0.020	4.45	0.091	0.004	4.45	0.016	0.002	4.45	0.008	0.001	4.45	0.004
11	0.014	4.45	0.064	0.002	4.45	0.009	0.001	4.45	0.004	<0.001	4.45	0.002
12	0.010	4.45	0.045	0.001	4.45	0.005	0.001	4.45	0.002	<0.001	4.45	0.001

Note: In scenario A (Hoenig), $S_0 = 0.475$, $S = 0.705$; in scenario B (Pauly), $S_0 = 0.475$, $S = 0.580$; in scenario C (LCCC-2), $S_0 = 0.475$, $S = 0.535$; and in scenario D (LCCC-1), $S_0 = 0.475$, $S = 0.498$. R_0, multiplication rate per generation; G, mean generation time; r, intrinsic rate of population increase corrected through the Euler equation (Wilson and Bossert 1971); $t_{\times 2}$, population doubling time (yr). For scenario A, $R_0 = 3.453$, $G = 5.08$, $r = 0.272$, and $t_{\times 2} = 2.5$. For scenario B, $R_0 = 1.686$, $G = 4.34$, $r = 0.125$, and $t_{\times 2} = 5.5$. For scenario C, $R_0 = 1.299$, $G = 4.13$, $r = 0.064$, and $t_{\times 2} = 10.8$. For scenario D, $R_0 = 1.043$, $G = 3.98$, $r = 0.011$, and $t_{\times 2} = 63.0$.

that the age at theoretical maximum size for females (115 cm TL in Tampa Bay and 103 cm TL in Florida Bay) was 12+ yr. In contrast, the theoretical maximum size of males (≈89 cm TL in Tampa Bay and ≈82 cm TL in Florida Bay) was only 8+ yr. In general, small temperate or subtropical shark species, such as the bonnethead shark, may be characteristically short lived compared with larger species or species inhabiting cooler waters. This trend is followed by the Atlantic sharpnose shark, another small temperate and tropical species, which reaches only 110 cm TL (Compagno 1984) and lives an estimated 8–10 yr (Branstetter 1987). In contrast, the spiny dogfish inhabits colder waters, reaches a maximum total size of ≈120 cm TL, and reportedly lives up to 70 yr (Jones and Geen 1977) or more (Saunders and McFarlane 1993). Likewise, other larger species, such as the lemon and sandbar sharks, reach at least 26 yr (Hoenig and Gruber 1990) and 35–50 yr (Casey and Natanson 1992), respectively.

Survivorship

Our estimate of 1st yr survivorship (0.475) for bonnethead sharks was derived from a multiple-census and removal study conducted by Manire and Gruber (1993) on an unexploited population of lemon sharks in their natural environment, a semi-enclosed, mangrove-fringed lagoon area in Bimini, Bahamas, in which it was fairly safe to assume a closed population. Because their calculated annual mortality rate might have been affected nevertheless by emigration, immigration, and net avoidance, these authors cautioned that the actual natural mortality rate lay probably between their calculated and minimum estimates, hence our use of 0.475 as an estimate of 1st yr survivorship. While this value of survivorship seems somewhat low for young-of-the-year lemon sharks, the situation for bonnethead sharks may be different. Lemon sharks are born at ≈60–65 cm TL (Compagno 1984) in well-protected shallow-water areas serving as nursery grounds during the first years of life (Morrissey and Gruber 1993), but bonnethead sharks are born at only ≈25–35 cm TL in less protected bodies of water. On the basis of their small size at birth and the habitat in which they are born, young-of-the-year bonnethead sharks are probably subjected to higher mortality by predators than young-of-the-year lemon sharks. Owing to the lack of information on mortality rates in sharks, it is difficult to evaluate whether the overall natural mortality is indeed higher in the bonnethead shark than in longer lived species, such as the lemon shark. Cortés (1995) found in a demographic analysis of the similarly short-lived Atlantic sharpnose shark that a 1st

Table 3. Survivorship and fertility tables for bonnethead sharks from Florida Bay, representing two scenarios based on two different values of survivorship for sharks of 1+ yr (S) and constant 1st yr survivorship ($S_0 = 0.475$).

	Scenario A			Scenario B		
	l_x	m_x	l_xm_x	l_x	m_x	l_xm_x
0	1.000	0	0	1.000	0	0
1	0.475	0	0	0.475	0	0
2	0.335	0	0	0.252	0	0
3	0.236	4.65	1.098	0.133	4.65	0.620
4	0.166	4.65	0.774	0.071	4.65	0.329
5	0.117	4.65	0.546	0.037	4.65	0.174
6	0.083	4.65	0.385	0.020	4.65	0.092
7	0.058	4.65	0.271	0.011	4.65	0.049
8	0.041	4.65	0.191	0.006	4.65	0.026
9	0.029	4.65	0.135	0.003	4.65	0.014
10	0.020	4.65	0.095	0.002	4.65	0.007
11	0.014	4.65	0.067	0.001	4.65	0.004
12	0.010	4.65	0.047	<0.001	4.65	0.002

Note: In scenario A (Hoenig), $S_0 = 0.475$, $S = 0.705$; in scenario B (Pauly), $S_0 = 0.475$, $S = 0.529$. R_0, multiplication rate per generation; G, mean generation time; r, intrinsic rate of population increase corrected through the Euler equation (Wilson and Bossert 1971); $t_{\times 2}$, population doubling time (yr). For scenario A, $R_0 = 3.608$, $G = 5.08$, $r = 0.283$, and $t_{\times 2} = 2.4$. For scenario B, $R_0 = 1.318$, $G = 4.11$, $r = 0.068$, and $t_{\times 2} = 10.2$.

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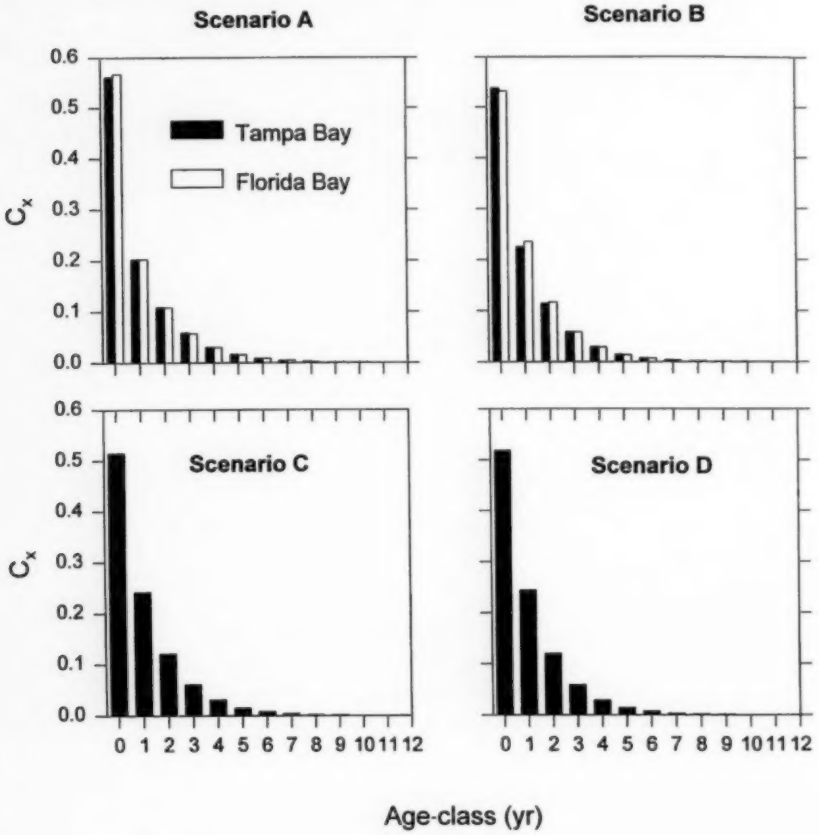
Fig. 2. Predicted stable age distributions (C_x) of bonnethead sharks under four scenarios in Tampa Bay and two scenarios in Florida Bay, assuming geometric population growth. See Tables 2 and 3 for definition of scenarios.



yr survivorship of 51% for pups born at ≈30–35 cm TL would only allow the population to remain at equilibrium ($r = 0$). First year survivorship values much lower than 50% are thus unlikely.

Our estimates of annual survivorship for age 1+ bonnethead sharks are among the lowest reported for any species of sharks. Our largest estimate ($S = 0.705$), derived using Hoenig's (1983) equation, is in good agreement with an estimate derived by Cortés (1995) for the Atlantic sharpnose shark ($S = 0.66$) using the same method. In using this method we assumed that Z equalled M, i.e., that F was negligible. However, any directed fishery or significant increase in by-catch from other fisheries for this species would result in smaller M values through Hoenig's equation and yield higher r values in the life tables. The lack of basic biological information on age or size composition for most species of sharks, especially the longer lived species, helps explain why most other demographic studies of shark populations have used this simplified approach to estimate mortality. Furthermore, since Hoenig's equation assumes a direct relationship between longevity (t_{max}) and survivorship, large values of annual survival were derived and used in demographic studies of long-lived species (Table 4). The lower survivorship values for the Atlantic sharpnose shark ($t_{max} = 10$) and the bonnethead shark ($t_{max} = 12$), two relatively short-lived species, are consistent with the smaller maximum size of these species (≈110 and 115 cm TL, respectively), which would make them more vulnerable to predation by other sharks, especially at earlier ages, given the small size of pups in these two species.

The estimates of annual survival for the bonnethead shark derived using Pauly's equation ($S = 0.580$ in Tampa Bay and 0.529 in Florida Bay) are lower than those derived using Hoenig's equation. The reliability of this method is related to the estimates of L_∞ and K from the VBGF and to an accurate estimate of mean annual water temperature. This method was also used by Hoenig and Gruber (1990) in their demographic analysis of the lemon shark, resulting in an estimate of $S = 0.87$ for $\overline{T} = 30°C$. We calculated annual survival through this method for the Atlantic sharpnose shark ($S = 0.58$ for $\overline{T} = 20°C$) and the leopard shark ($S = 0.86$ for $\overline{T} = 15°C$), finding values close to those obtained through Hoenig's equation (Table 4). It should be kept in mind that both Hoenig's and Pauly's equations were developed from teleost fishes, which probably have higher mortality values than sharks. However, if a similar equation developed by Hoenig (1983) for cetaceans

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Fig. 3. Mean expectation of further life (e_x) as a function of age for female bonnethead sharks from Tampa Bay (●, scenario A; ■, scenario B; ▼, scenario C; ▲, scenario D) and Florida Bay (●, scenario A; □, scenario B). See Tables 2 and 3 for definition of scenarios.

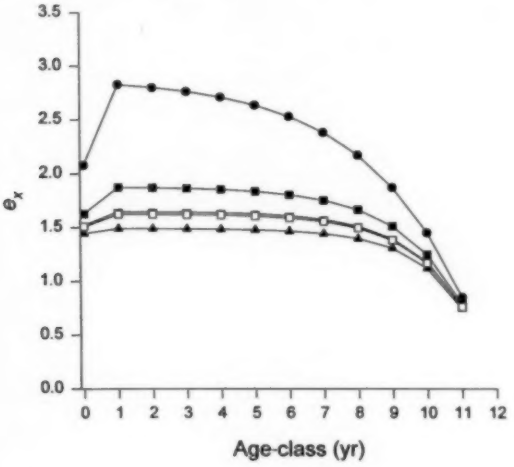
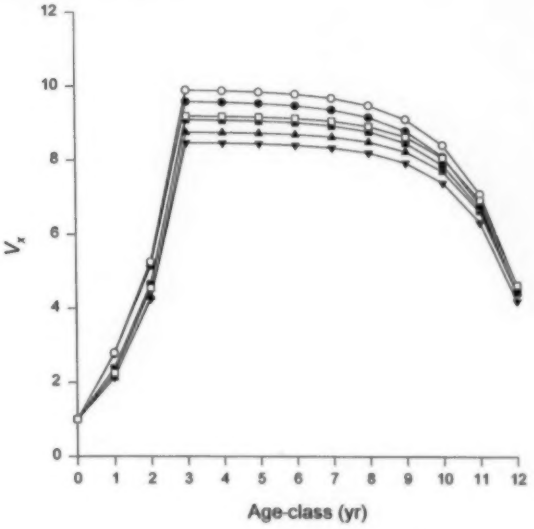


Fig. 4. Reproductive value (V_x) as a function of age for female bonnethead sharks from Tampa Bay (●, scenario A; ■, scenario B; ▼, scenario C; ▲, scenario D) and Florida Bay (○, scenario A; □, scenario B). See Tables 2 and 3 for definition of scenarios.



is used to calculate Z for the bonnethead shark, survivorship is only increased by about 6%.

Our lowest estimates of annual survival for the bonnethead shark were obtained through two similar length-converted methods, which we could only apply to the Tampa Bay population. These methods assume that mortality, or survivorship, is constant in all age groups used in the calculation. Although no age-specific mortality rates are available for any shark species, a near constant survivorship in adult age-classes has been found in studies of other vertebrates (Ricker 1975; Frazer et al. 1991). Since our calculations included mostly mature sharks and given the relatively good fit of the linear regressions (Table 1), it seems reasonable to assume that survivorship was nearly constant. In contrast, Walker (1994*b*) replaced the usual assumption of constant natural mortality by using a U-shaped function to vary natural mortality with age in his fishery model of the gummy shark in the Bass Strait, Australia. The length-converted methods also assume no gear selectivity for the age groups used in the calculation. While all bonnethead sharks older than 1 yr are vulnerable to the gill nets used, only carefully designed experiments using gill nets of different mesh sizes can determine whether all sharks are equally vulnerable to the fishing gear. Finally, the length-converted methods assume constant year-to-year recruitment. Since the early work by Holden (1974, 1977), it has been believed that parental stock and recruitment in elasmobranchs are almost linearly related, implying that any significant change in the number of reproductive females would affect the number of progeny. Recently, Walker (1994*b*) reported a stock–recruitment relationship for the gummy shark resembling the Beverton–Holt curve (Beverton and Holt 1957). While there is some evidence that the fecundity of the spiny dogfish in Scottish–Norwegian waters responded to changes in stock abundance (Holden 1977), we need a much better understanding of the extent to which the reproductive biology of elasmobranch fishes is affected by variations in stock size through density-dependent mechanisms. In the case of the bonnethead shark, no data on year-class strength are available, but since fishing mortality is negligible, barring any large environmental fluctuations that would affect population size, recruitment can be assumed to be fairly constant from year to year. The assumptions implicit in the length-converted methods thus appear to be reasonably well fulfilled for the bonnethead shark.

The high mortality values derived for this species through the length-converted methods raise the issue of their reliability and of that of past demographic studies of shark species that only used Hoenig's equation to estimate mortality. From our results, it is apparent that using length-converted methods will yield higher mortality values, which will be biased upwards by emigration and downwards by immigration. It is thus important that future demographic studies of shark species attempt to use several methods to estimate mortality because as we have seen in the present study results can vary radically.

Life tables

Except for the revised demography of the spiny dogfish (G.M. Cailliet, Moss Landing Marine Laboratories, Moss Landing, P.O. Box 450, CA 95039, U.S.A., personal communication) and the fishery models for gummy shark in Australia (Walker 1994*a*, 1994*b*), all other demographic studies of shark species have assumed that the totality of mature females are reproductively active every breeding season (Hoenig and Gruber 1990; Hoff 1990; Cailliet 1992; Cailliet et al. 1992; Cortés 1995). While we recognize that our estimates of fecundity may be somewhat high, there is evidence that most mature female bonnethead sharks caught during the breeding season in Tampa Bay are indeed pregnant (G.R. Parsons, personal observation; Manire et al. 1995). Future demographic analyses

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Table 4. Longevity (t_{max}), annual survivorship (S), and demographic parameters for several shark species compared with values derived in this study for bonnethead sharks from southwest Florida.

	t_{max}	S	R_0	G	r	$t_{\times 2}$	Source
Triakis semifasciata	30	0.87	4.47	22.35	0.067	10.3	Cailliet 1992
Negaprion brevirostris	26	0.85	1.27	16.23	0.015	46.7	Hoenig and Gruber 1990
Squalus acanthias	70	0.91	3.05	49.63	0.023	30.1	Jones and Geen 1977[a]
Carcharhinus plumbeus	35	0.90	1.48	21.5	0.018	38.5	Hoff 1990
Squatina californica	35	0.82	2.25	14.5	0.056	12.4	Cailliet et al. 1992
Rhizoprionodon terraenovae	10	0.66	1.28	5.78	0.044	15.7	Cortés 1995[b]
Sphyrna tiburo	12	0.70	3.61	5.08	0.283	2.4	This study[c]
	12	(0.50)	(1.04)	(3.98)	(0.011)	(63.0)	

[a]Recalculated by T. Eguchi and G.M. Cailliet (Moss Landing Marine Laboratories, P.O. Box 450, Moss Landing, CA 95039, U.S.A., personal communication).

[b]Best case scenario.

[c]Based on maximum estimates (minimum estimates in parentheses).

of shark species should routinely attempt to investigate ontogenetic variations in the proportion of mature females reproductively active each year or breeding season and the effect of senescence on fertility rates.

Like other demographic studies of shark populations, our estimates of demographic parameters obtained through life tables do not incorporate possible emigration and immigration of individuals in the populations under study. Ongoing tagging studies along the west coast of Florida should help elucidate how much emigration and immigration affect population numbers. Likewise, our estimates may be affected by the inability of demographic analyses to incorporate compensatory changes in survivorship and fecundity with time. Thus, our estimates should be regarded as a snapshot of the two populations at the time the data were gathered. The populations studied seem to be in an increasing phase because they may not have reached the environmental carrying capacity and abiotic and biotic conditions may be favorable. Certainly, this situation will not last indefinitely, and when the populations reach their maximum size as permitted by the environment, density dependence will come into play and bring the populations back to near-equilibrium. Likewise, if environmental or ecological conditions become unfavorable, the populations may enter a decreasing phase. Compensatory mechanisms affecting population sizes in the bonnethead and other shark species may include changes in growth rate, age at maturity, fecundity, rates of emigration and immigration, and survival rates through density-dependent regulation.

Our estimates of the intrinsic rate of population increase and other demographic parameters (R_0, G, $t_{\times 2}$) for the two populations of bonnethead sharks suggest that population growth is slightly higher in Florida Bay than in Tampa Bay. However, this is probably due to the values of annual survival used in the calculation of the life tables. We believe that had we been able to apply the length-converted methods to estimate survivorship in the Florida Bay population, we would have obtained lower values of population growth, reflecting the life history characteristics of that subtropical population of bonnethead sharks. Furthermore, if we consider only Pauly's equation, the rate of population increase is 13%/yr in Tampa Bay and only 7%/yr in Florida Bay.

As a species, population growth in the bonnethead shark appears to be considerably faster than in other sharks if we consider the most optimistic scenario (Table 4). Our estimates of G varied from ≈5 to 4 yr and were only comparable with that derived for the similarly short-lived Atlantic sharpnose shark (G = 5.8 yr). The other demographic parameters (R_0, r, and e^r) can all be expressed as the time it takes a population to double in numbers ($t_{\times 2}$). Using our most optimistic estimates, bonnethead shark populations would double every ≈2.5 yr, whereas it would take 63 yr for those same populations to double in size under the most pessimistic scenario. We feel that the most likely situation is closer to our most optimistic scenario and that bonnethead shark populations grow at a very high rate compared with other species of sharks. The high abundance of this species in shallow coastal areas along the west coast of Florida seems to support our hypothesis and suggests that the bonnethead shark is probably one of the most abundant species of sharks in the southeastern U.S.A.

It is interesting from an ecological perspective to compare the life table information on the bonnethead shark (Sphyrnidae) with that of another small coastal species with similar life history characteristics, the Atlantic sharpnose shark (Carcharhinidae). Cortés (1995) found that populations of Atlantic sharpnose sharks in the Gulf of Mexico increased at a rate of 4.5%/yr ($e^r = e^{0.044} = 1.045$) under his best case scenario using Hoenig's equation (Table 4). In our best case scenarios for Tampa and Florida bays (also Hoenig's equation), we found that the populations could be increasing at a rate greater than 30%/yr. Only in our worst case scenario for Tampa Bay would the bonnethead shark population be increasing at a rate of ≈1%/yr. The difference in population rate of increase between these two species, which occupy similar habitats, at least during part of their life history, is a result of differences in survivorship, fecundity, age at maturity, and longevity.

Atlantic sharpnose sharks are born at ≈30–35 cm TL in coastal waters about 10 m deep (Parsons 1983; Castro 1993), reaching a maximum size of at least 110 cm TL (Compagno 1984), while bonnethead sharks are born at ≈25–35 cm TL (Parsons 1993*b*), probably in shallower waters, and individuals from some populations are reported to reach a maximum size of at least 130 cm TL (Compagno 1984). Numerous biotic and abiotic factors, including habitat type, behavior and activity patterns, and presence and type of predators, may interact with each other and with body size to determine the vulnerability of newborn and older sharks to predation or other sources of mortality. While it is still speculative at this point, our suggestion is that the small size of Atlantic sharpnose

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sharks, combined with the relatively deeper waters in which they generally occur, may result in higher mortality for this species on the basis of size-selective predation only, all other factors being approximately equal.

Fecundity in the Atlantic sharpnose shark increases with female size (Parsons 1983; Cortés 1995) but is lower than in female bonnethead sharks. Furthermore, female Atlantic sharpnose sharks mature at a later age than female bonnethead sharks (4 versus 3 yr) and have a shorter life-span (10 versus 12 yr), which means that female Atlantic sharpnose sharks are reproductively active for only 7 yr compared with 10 yr for female bonnethead sharks. Thus, decreased survivorship and fertility schedules in the Atlantic sharpnose shark would combine to produce slower population growth in that species than in the bonnethead shark.

Intraspecific comparisons of life history traits of populations from different environments are instructive regarding how various selective pressures affect life histories (Baltz 1990). Comparisons of life tables for populations of the same species in different locations or environments or for similar species in the same or similar environments can offer insight into life history strategies and how environmental variability and phylogenetic relationships shape demographic parameters. While previous studies on reproduction and age and growth established marked differences in life history characteristics between the two bonnethead shark populations from Tampa Bay and Florida Bay, the present study was inconclusive concerning distinct evidence of differences in life table statistics of the two populations. Furthermore, the role of environmental variability and heritability in shaping demographic parameters remains to be determined. Future studies of shark populations should investigate the intraspecific variability in life history characteristics and how these affect the demography of different populations of the same species.

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Juvenile sockeye rearing capacity of three lakes in the Fraser River system

Jeremy M.B. Hume, Ken S. Shortreed, and Ken F. Morton

Abstract: We used three methods to estimate sockeye salmon (*Oncorhynchus nerka*) escapements that maximize production (S_{MAX}) in Fraser River lakes: (*i*) effective female spawners and adult returns using Ricker stock-recruit analysis (SR models), (*ii*) effective female spawners and fall fry or smolts (fry models), and (*iii*) photosynthetic rates (PR model), a modification of an Alaskan sockeye production model (EV model). Adult SR models were not useful in predicting S_{MAX} because of high variability in Shuswap and Chilko lakes ($r^2 < 11\%$) and because of linearity in Quesnel Lake. Fry models using Ricker stock-recruit analysis provided S_{MAX} escapements of 0.90, 1.38, and 1.06 million for Quesnel, Shuswap, and Chilko lakes but were still highly variable ($r^2 < 51\%$). Fry data indicated that fry numbers did not increase above escapements of 0.85, 1.5, and 0.51 million to Quesnel, Shuswap, and Chilko lakes. PR model predictions of S_{MAX} escapements to Quesnel, Shuswap, and Chilko lakes of 1.06, 1.85, and 0.62 million were similar to escapements that first produced maximum observed fry numbers. While fry models provide a direct estimate of rearing capacity, many years of data are required to generate a relationship for any lake. The PR model appears to be a useful predictor of rearing capacity and predictions can be made after 1–2 years.

Résumé : Nous avons utilisé trois méthodes pour estimer les échappées de saumons sockeye (*Oncorhynchus nerka*) en vue de maximiser la production (S_{MAX}) dans les lacs du fleuve Fraser : (*i*) retours des géniteurs femelles efficaces et des adultes par analyse des stocks-recrues à l'aide de la technique de Ricker (modèles SR), (*ii*) les géniteurs femelles efficaces et alevins ou saumoneaux d'automne (modèles des alevins) et (*iii*) taux de photosynthèse (modèle PR), une variante d'un modèle de production de sockeye d'Alaska (modèle EV). Les modèles SR des adultes n'étaient guère utiles pour prévoir la valeur de S_{MAX}, en raison de la grande variabilité des lacs Shuswap et Chilko ($r^2 < 11\%$) et de la linéarité dans le lac Quesnel. Les modèles d'alevins faisant appel à l'analyse des stocks-recrues à l'aide de la technique de Ricker a donné des échappées S_{MAX} de 0,90, 1,38 et 1,06 million pour les lacs Quesnel, Shuswap et Chilko, mais ces valeurs étaient encore très variables ($r^2 <$ 51%). Les données sur les alevins indiquaient que leur nombre n'augmentait pas au-dessus des échappées de 0,85, 1,5 et 0,51 million pour les lacs Quesnel, Shuswap et Chilko. Les prévisions du modèle PR pour les échappées S_{MAX} vers les lacs Quesnel, Shuswap et Chilko, qui étaient de 1,06, 1,85 et 0,62 million, étaient semblables aux échappées qui avaient d'abord donné les nombres maximum d'alevins observés. Même si les modèles d'alevins permettent d'estimer directement la capacité d'alevinage, des données obtenues sur plusieurs années sont nécessaires pour dégager une relation pour un lac quelconque. Le modèle PR semble constituer un prédicteur utile de la capacité d'alevinage, et il est possible de faire des prévisions après 1 ou 2 ans.
[Traduit par la Rédaction]

Introduction

Sockeye salmon (*Oncorhynchus nerka*) are the most economically valuable salmon species in British Columbia, with the value of the annual catch often exceeding 100 million dollars. The Fraser River is the world's largest single river producer of sockeye salmon, with recent adult returns exceeding 15 million in some years. It is surpassed only by the combined sockeye production from several river systems flowing into Bristol Bay in Alaska (Northcote and Larkin 1989). Most Fraser River sockeye smolt after 1 year of lake residence and return to spawn in their 4th year after 2+ years at sea (age 4_2). There is often a 4-year cycle of abundance, with one dominant return, one much smaller subdominant return, and two extremely small nondominant returns (Fig. 1). The cause(s) of this is (are) unknown, but proposed explanations have ranged from biological interaction between brood years (Ricker 1950; Ward and Larkin 1964) to high fishing rates (Walters and Staley 1987). Commercial catch of Fraser River sockeye peaked in 1913, when 30 million fish were harvested, but shortly thereafter stocks precipitously declined owing to the combined influence of overfishing and blockage of the river during railway construction in the Fraser Canyon (Thompson 1945; Ricker 1947). The size of some Fraser sockeye stocks is currently increasing, but production of the whole system has not yet attained the estimated historic production of 100 million fish in dominant years (Ricker 1987). The Fraser system's exceptionally high productivity is due to the presence of many large lakes that are accessible to anadromous fish. Further, most of these lakes are sufficiently productive to sustain a zooplankton community capable of supporting juvenile sockeye

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J.M.B. Hume[1] **and K.F. Morton.** Department of Fisheries and Oceans, Cultus Lake Laboratory, 4222 Columbia Valley Highway, Cultus Lake, BC V2R 5B6, Canada.
K.S. Shortreed. Department of Fisheries and Oceans, West Vancouver Laboratory, 4160 Marine Drive, West Vancouver, BC V7V 1N6, Canada.

[1] Author to whom all correspondence should be addressed.

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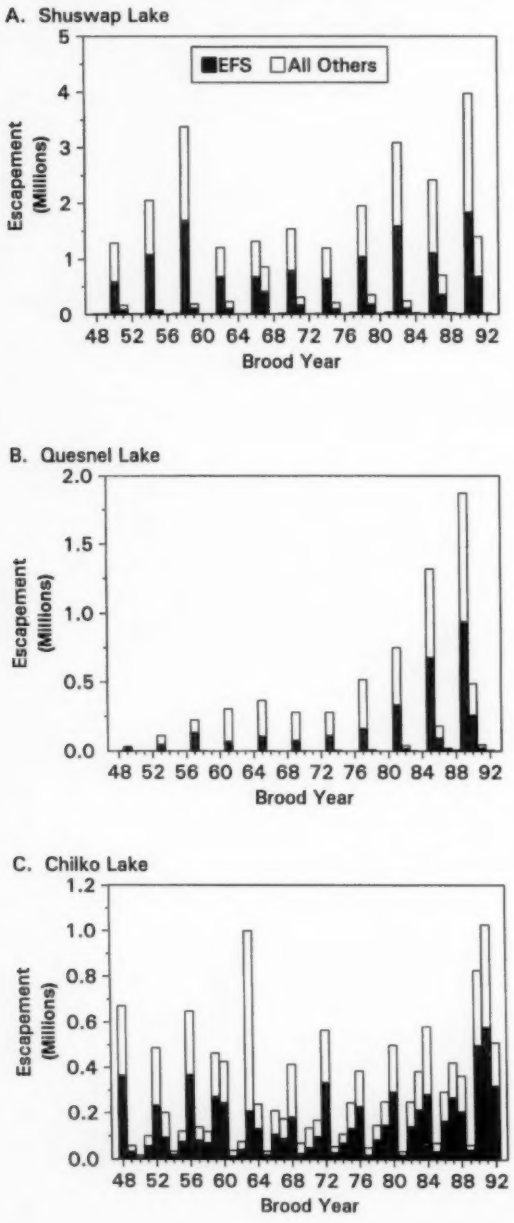
Fig. 1. Escapements to Shuswap, Quesnel, and Chilko lakes showing effective female spawners (EFS, solid bars) and all other spawners (males, jacks, and other females).



densities far higher than presently occur (Stockner and Shortreed 1983).

Because most Fraser sockeye stocks were recruitment limited for most of this century, increasing escapements within the constraints imposed by the commercial fishery have been a primary goal of Fraser River sockeye managers. Owing to uncertainties about the cause of the 4-year cycle of abundance, these rebuilding efforts have been largely restricted to the dominant and (to a lesser extent) subdominant cycle years. The rebuilding efforts have been successful on some Fraser system lakes, and particularly so on the lakes in this study. During the rebuilding period, when escapements were relatively low, rearing capacity of the lakes was not a concern. Rather, optimum escapement estimates were based on estimates of spawning ground capacity (Roos 1989). Since the 1980s in Shuswap and Quesnel lakes and 1990 in Chilko, dominant and subdominant brood year returns and escapements have been very high (Fig. 1, the 1958 return to Shuswap Lake was also high, but subsequent returns dropped considerably and have been building ever since). Determination of escapement levels that will maximize subsequent adult returns is now crucial to the efficient management of Fraser sockeye stocks. Escapements lower than the optimum will result in reduced adult returns. In any brood year, escapements higher than the optimum entail foregoing harvestable sockeye and will produce (at best) no increases in harvestable sockeye in subsequent brood years. If high escapements result in excessive fry recruitment and if the high escapements are consecutive, substantial and long-term declines in total stock size (Kyle et al. 1988) may occur, resulting in considerable economic loss.

Since the mid 1980s we have been conducting studies on these three lakes. Our studies are the first that have included detailed investigations of every major lake trophic level (from the microbial community to planktivorous fish) as well as measurement of salient physical and chemical variables. This ecosystem approach has enabled us to produce the first estimates of optimum spawning escapements based on a lake's productivity and on its ability to rear juvenile sockeye. The objectives of this study were twofold. First, we calculated and compared the size of the optimum escapements to each of the three lakes as predicted by adult sockeye stock-recruitment data and as predicted by juvenile sockeye freshwater size and abundance data. Second, utilizing pertinent limnological data, we modified a rearing capacity model developed by Koenings and Burkett (1987). This modified model enabled us to predict escapements that will maximize smolt output from a lake. We compared these predictions with observed maximum juvenile sockeye production from our study lakes. Calculated maximum smolt production and escapements that produce these maxima vary considerably among the three lakes, and we present salient limnological data to explain these differences.

Description of study lakes

Chilko, Quesnel, and Shuswap lakes are located in the south-central portion of the interior plateau of British Columbia and are three of the larger lakes in the Fraser River drainage basin (Fig. 2). The climate consists of cold winters and warm, dry summers but winters are much less severe at Shuswap than at Chilko Lake.

Quesnel Lake (52°30′N, 120°00′W) has a surface area of 270 km^2 and its maximum depth of 530 m (mean depth = 158 m) makes it the ninth deepest lake in the world. It lies at an elevation of 725 m in the Cassiar-Columbia Mountain physiographic region and the Interior Western Hemlock biogeoclimatic zone of central British Columbia (Farley 1979). Annual precipitation ranges from <75 cm at the western end of the lake to about 150 cm at the eastern end (Farley 1979). The lake has a large (5930 km^2) drainage basin and a water

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Fig. 2. Map of British Columbia showing the location of the three study lakes and other British Columbia lakes mentioned in the text.

residence time of 10.8 years (Stockner and Shortreed 1983). Quesnel Lake is dimictic, with winter ice cover occurring each year. The most important spawning areas for adult sockeye are the lake's two major tributaries, the Horsefly and Mitchell rivers, while smaller numbers of sockeye spawn in several small streams (Morton and Williams 1990). Further, unknown (but assumed to be small) numbers spawn along the shores of the lake. Two shallow bays have very low densities of sockeye fry and have been excluded from our analysis of sockeye densities (adults and juveniles), resulting in a surface area of 261 km^2.

Chilko Lake (51°20′N, 124°05′W) is situated at an elevation of 1172 m at the boundary of the coast mountain range and the interior plateau. The 185-km^2 lake is located in the Cariboo aspen – lodgepole pine and subalpine Engelmann spruce – subalpine fir biogeoclimatic zones (Farley 1979). Annual precipitation averages <100 cm. The steep-sided nature of the surrounding terrain and of the lake itself results in an extremely limited littoral area. The mean depth of the lake is 123 m, the maximum depth is approximately 330 m, and the area of the drainage basin is 2734 km^2. In most winters complete ice cover does not occur on Chilko Lake, but winter water temperatures are <4°C, with substantial vertical mixing occurring all winter, and it therefore cannot be classified as warm monomictic (Hutchinson 1957). Stockner and Shortreed (1991) classified Chilko Lake as cool monomictic (one period of circulation each year at temperatures <4°C). During our study (1984–1993) water residence times averaged 21 years. During summer, the southern portion of Chilko Lake receives several glacially turbid inflows, with the result that water clarity tends to decrease both spatially from north to south and seasonally from May to August. The orientation of Chilko Lake at the edge of the coast mountains results in frequent strong southerly catabatic winds. These winds exert a strong influence on the circulation patterns and thermal structure of the lake. Over one half of the total sockeye escapement to Chilko Lake spawns in the Chilko River, which is the lake outlet. Spawning occurs primarily in 2 km of river immediately

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below the lake outlet. A variable number (ranging from 10 to 50% of the total escapement) spawn in the lake, utilizing beaches with suitable gravel at locations scattered throughout the lake.

Shuswap Lake, with an area of 330 km^2 (including Little Shuswap and Mara lakes), is the largest in the Fraser River system. It is the most southerly (50°00′N, 119°05′W) of our study lakes and also is situated at a substantially lower elevation (347 m). Consequently, the climate is milder, resulting in a longer growing season, more prolonged and pronounced thermal stratification, and a warmer epilimnion than in Chilko or Quesnel lakes. Annual precipitation is 50–75 cm (Farley 1979). The large drainage basin (16 221 km^2) and relatively shallow depth (mean = 62 m, maximum = 162 m) results in a water residence time of only 2.1 years. While occasional strong winds do occur, the climate at Shuswap Lake is much less windy than at either Quesnel or Chilko lakes. Sockeye spawn in a number of lake tributaries and in the lake itself, with approximately 70% of the total escapement spawning in the Adams River, which enters Shuswap Lake only 2 km from the outlet of the lake. As sockeye fry occur only in low densities in the extensive shallows found in parts of the lake, we have excluded these areas in our analysis of sockeye densities (adults and juveniles), resulting in a surface area of 315 km^2.

Methods

Adult sockeye

Total adult escapements, numbers of effective female spawners (EFS), and total returns have been estimated from 1948 to 1994 for the three study lakes (Cass 1989; Roos 1989; Pacific Salmon Commission 1993; W. Saito and T. Whitehouse, Department of Fisheries and Oceans, New Westminster, B.C., personal communication). We used data from the 1948 to 1991 brood years for EFS data and from the 1948 to 1988 brood years (1992 return year) for return data. Only age-4$_2$ adult returns are used in this analysis. Age-4$_2$ sockeye account for 93, 98, and 92% of total returns to Shuswap, Quesnel, and Chilko lakes, respectively. Numbers of EFS are widely used in stock-recruitment analyses and are used here as estimators of fry recruitment. EFS are female sockeye that have successfully spawned, as determined by examination of carcasses on the spawning grounds. We make the assumption that the number of EFS is directly related to fry entering the lake from the spawning grounds (we have no data on variation in egg to fry survival). EFS averaged 95% of total females (range 87–100%) in Shuswap, 81% (range 15–100%) in Quesnel, and 90% (range 38–100%) in Chilko lakes. High prespawning mortality caused the low proportions of EFS in Quesnel and Chilko lakes in some years, and was attributed to returning adults being exposed to high water temperatures both during migration and on the grounds (Gilhousen 1990). In Shuswap Lake, EFS averaged 53% of total escapements of age-4$_2$ adults, but ranged widely from 45 to 68%. EFS in Quesnel and in Chilko lakes averaged 46 and 54% of adult escapement, respectively, with an even greater range (23–65% in both lakes).

A number of authors have analyzed stock-recruitment relationships in Fraser River sockeye populations to examine either optimum harvesting strategies or the 4-year cycle phenomenon seen in some of these stocks (Ward and Larkin 1964; Larkin 1971; Collie and Walters 1987; Cass 1989; Collie et al. 1990; Welch and Noakes 1991). Much of this analysis has been done by applying the Ricker (1975) stock-recruit model to various sockeye stocks. The form of the model commonly used for Pacific salmon is

$$R = Se^{a(1-S/b)} + \varepsilon$$

where R is total returns (catch and escapement), S is the EFS, e^a is the initial slope of the curve, b is the value of S where $R = S$, and ε is the error term. a is a measure of the stock growth rate or productivity and b is a measure of the capacity of the stock. Both a and b affect the typical parameters of interest in a stock-recruit analysis: the escapement (S_{MSY}) that maximizes the return to the fishery, and the escapement (S_{MAX}) that maximizes total return. For the purpose of estimating rearing capacity, S_{MAX} is the more appropriate parameter.

A common way of fitting a set of data to the Ricker model is by transforming the equation into the following form:

$$\ln\left(\frac{R}{S}\right) = a - \frac{a}{b}S + \varepsilon$$

and treating it as a linear regression. Calculation of variance and tests of significance are straightforward in the transformation and provide a good indicator of the reliability of the model's fit to the data (Hilborn and Walters 1992).

Juvenile sockeye

Juvenile sockeye numbers and size have been determined with hydroacoustic and trawl surveys from Quesnel and Shuswap lakes since the mid 1970s (Williams et al. 1989; Hume et al. 1994). All of our sampling was done during the hours of darkness, when the fish were dispersed and within the working range of the midwater trawl and hydroacoustic system (McDonald and Hume 1984; Burczynski and Johnson 1986; Levy 1990). Data were collected from Shuswap Lake in the year following spawning for 6 of the 12 brood years from 1974 to 1985 and for every brood year from 1986 to 1991. Quesnel Lake data were collected for 4 dominant brood years since 1977 and for all brood years from 1985 to 1991. Hydroacoustic and trawl data were collected in the summer (late July and August) or in the fall (October and early November). Smolt size data are available in Quesnel and Shuswap lakes for some dominant years since 1961. An appropriate time series of hydroacoustically derived juvenile sockeye population estimates is unavailable for Chilko Lake. However, smolt numbers and size have been determined at Chilko Lake for the brood years from 1949 to 1992 (1951–1994 smolt years), except for the 1989 brood year when only size data were collected (Roos 1989; T. Whitehouse, personal communication). Consequently, we used smolt rather than fall fry data in our Chilko Lake analyses.

Prior to hydroacoustic surveys, lakes were divided into a number of sections on the basis of lake morphometry. Within each section, two to three evenly spaced hydroacoustic transects were established. There were a total of 16 transects on Quesnel Lake and 33 on Shuswap Lake. The same transects were used on all surveys since 1975. Results from each transect were used to provide a mean estimate of density (number per hectare) for each lake section. The mean density was then multiplied by the surface area of the section to provide a population estimate for the section and then summed to provide a total population estimate for the lake. Mean lake density was calculated by dividing the lake population estimate by the total surface area. Variances were calculated for the density of each section and were then weighted by the square of the section area. The sum of the weighted variances was divided by the square of the lake area to provide a variance for the lake population estimate. In this paper we report 2 times the standard error.

From 1974 to 1984, acoustic data were collected using a Simrad EY-M echosounder with a 70-kHz transducer producing an 11° beam (at –3dB) and recorded for later processing. Data were analyzed in two stages with the duration in beam technique (Thorne 1988). First, recorded voltages were integrated with a Biosonics 121 integrator to give the relative uncalibrated density of fish in each transect. Second, targets were counted on an oscilloscope from selected transects in each lake. These counts were then regressed against the integrated data from the same transect. The regression line was then used to calibrate all of the integrated transects to provide a density estimate for each transect.

From 1985 until 1994, data were generated with a Biosonics model

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105 dual beam echosounding system with a 420-kHz dual beam (6°/15°) transducer and were digitally recorded for later processing. Data were processed as described by Burczynski and Johnson (1986). First, target strengths and mean backscattering cross sections were determined for each transect with a Biosonics model 121 dual-beam processor. Second, recorded data were echo integrated to give the relative density of targets. Target strength and equipment scaling factors were then used to scale the echo integration to provide an estimate of fish density in each transect. In 1986 on Quesnel Lake we carried out concurrent surveys with the Simrad and Biosonics equipment to test comparability of data. There was only a 4% difference in juvenile sockeye population estimates generated by the two types of equipment and methods.

Fish samples were collected from each lake section with a 7 × 3 m midwater beam trawl (maximum mesh size 10.2 cm, fished at 1.0 m/s) as described by Enzenhofer and Hume (1989). Trawls of 5–45 min were made at locations and depths suggested by fish targets on the echosounder. Trawl duration was chosen to give an adequate sample size for later analysis (100–200 fish). All fish were anaesthetized and killed upon capture with an overdose of 2-phenoxyalcohol and then preserved in 10% formalin. Fish were kept in formalin for at least 1 month before lengths and weights were recorded. Trawl data were used to determine the species and age composition of the limnetic fish community. Smolt samples were preserved and analyzed in a similar manner.

Resident populations of kokanee (landlocked *O. nerka*) complicate the interpretation of size and abundance data in both Shuswap and Quesnel lakes. Precise abundance estimates are unavailable as no spawning ground counts are done and the age-0 class is visually indistinguishable from similarly sized sockeye. Parkinson et al. (1994) compared catches of kokanee by three otter trawls (maximum mesh size 3.2 cm, two fished at 1.5 m/s, one fished at 1.0 m/s) and by a beam trawl similar to ours (maximum mesh size 10.2 cm, fished at 1.0 m/s). They found little difference between the four trawls in length-frequency histograms of the catch of age-0 and -1 kokanee up to 155 mm in length, but the two slower trawls caught fewer larger fish (age-2 and -3 kokanee) than the two fast otter trawls. The mean size of each age-class in the beam trawl catch was always midway between that of the two fast otter trawls, indicating little relative bias in estimating mean age at length using the beam trawl. In our lakes, juvenile sockeye are predominantly age 0 and do not exceed 100 mm. The relatively small numbers of age-1 sockeye do not exceed 150 mm. We conclude that bias in our trawl data is restricted to underestimates of proportions of older kokanee (age-2 and -3). Estimates of mean size of each age-class appear to be unbiased. On the basis of known electrophoretic differences between adult kokanee and sockeye in Shuswap Lake, age-0 kokanee constituted 5% of the 1987 subdominant population and 73% of the 1989 nondominant population (C.C. Wood, J.M.B. Hume, and C.J. Foote, unpublished data). In Quesnel Lake, estimates of fall fry during nondominant years have ranged from 4 to 471/ha. A large but unknown proportion of these fish would be age-0 kokanee. In dominant years where fall fry estimates range from 1886 to 2561/ha, the proportion of age-0 and older kokanee would be small. Kokanee are rare in Chilko Lake and we have not caught any *O. nerka* older than age-1 in our trawls.

Limnological data

On Quesnel Lake, sampling for all limnological variables was carried out from 1985 to 1988 and again in 1990, except that photosynthetic rates (PR) were not determined in 1985. Data were collected once monthly (May–October) from six locations on the lake. The Shuswap Lake sampling program commenced in 1987 and concluded in 1993. At Shuswap Lake, PR was determined in every year except 1993. Each year sampling was carried out from March or April until November at locations in each of the four major lake basins. Sampling frequency was once monthly for most of the study, except for 1990 and 1991, when spring (April–June) sampling was carried out every 2 weeks. Sampling on Chilko Lake was carried out in May–October from 1984 to 1986 and from 1988 to 1993. Frequency of sampling ranged from once weekly to once monthly depending on the year and the sampling location. Numbers of locations were sampled, but for this study we used data collected from four sites spaced evenly along the lake's longitudinal axis. To calculate seasonal averages we defined the growing season in Chilko and Quesnel lakes as May 1 to October 31. This represented the period of active growth in the phytoplankton and zooplankton communities. Shuswap Lake's growing season was longer (April 1 to November 30). Time-weighted means for each sampling location were calculated by integrating seasonal data and dividing by the length of the growing season or other period of interest. Lakes were divided into zones representing major lake basins, and data from sampling locations within zones were averaged. Seasonal means for each zone were then weighted by the area of the zone and combined with data from other zones to yield a mean whole-lake value. Seasonal means of limnological data (except for zooplankton data) presented in this report are averages for the entire growing season. Zooplankton data are presented as July–November averages in Shuswap Lake and July–October averages in Chilko and Quesnel lakes to better represent the portion of the growing season when juvenile sockeye occupied the limnetic zone (Morton and Williams 1990; J.M.B. Hume, unpublished data).

We used Applied Microsystems Ltd. meters (models CTD-12 and STD-12) to measure conductivity, temperature, and depth. Photosynthetic photon flux density (PPFD: 400–700 nm) was determined with a Li-Cor model 185A light meter equipped with a model 192S underwater quantum sensor. Vertical light extinction coefficients and compensation depths were calculated. The compensation depth is the depth to which 1% of the surface light penetrates and we assumed that euphotic zone depth (EZD) was equivalent to compensation depth. We used an opaque 6-L Van Dorn water sampler to collect all water. Water from 5–9 depths from the surface to below the EZD was collected for analysis of chemical variables, phytoplankton biomass, and in situ PR. A 50 m deep water sample was also collected from each sampling location and analyzed for selected chemical variables. All chemical analyses followed methods described by Stephens and Brandstaetter (1983) and Stockner and Shortreed (1985). Water for dissolved nutrient analyses was filtered through ashed Whatman GF/F filters that were rinsed with both distilled deionized water and sample water immediately before filtration. We stored filtered water in clean glass and polyethylene bottles that were kept cold and dark before analysis. Nitrate was determined by converting to nitrite by cadmium reduction. Water for total phosphorus (TP) analysis was collected in clean, screw-capped glass test tubes, and TP was later determined with a molybdenum blue method after persulfate digestion. Samples for chlorophyll *a* (CHL) determinations were collected on Millipore AA filters (0.8-μm nominal pore size), frozen, and later analyzed fluorometrically after maceration in 90% acetone. PR was determined in situ by filling 125-mL glass bottles with water from each sampling depth, inoculating with a sodium bicarbonate solution containing ^{14}C, and incubating for 1.5–2.0 h at the original sampling depths. Incubations commenced between 09:00 and 10:00. After incubations, samples were filtered onto 0.2-μm Nuclepore filters, placed in scintillation vials containing a tissue solubilizer and scintillation cocktail, and counted in a Packard scintillation counter. Dissolved inorganic carbon concentrations were determined with the potentiometric method of the American Public Health Association, the American Water Works Association, and the Water Pollution Control Federation (1980). Hourly PR was calculated using Strickland's (1960) equation. Daily PPFD was determined with either a Li-Cor model 550 printing integrator (1984–1989) or a Li-Cor model LI-1000 data logger (1990–1993), each equipped with a model 190S quantum sensor. These data were then used to compute daily PR.

Zooplankton samples were collected with a 100-μm mesh size SCOR-UNESCO (SCOR) net (mouth area 0.25 m^2) hauled vertically

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from 50 m to the surface at Chilko Lake throughout the study, at Quesnel Lake from 1985 to 1987, and at Shuswap Lake in 1987. All other samples were collected with a 160-μm mesh size Wisconsin net (mouth area 0.05 m^2) hauled from 30 m to the surface. In our study lakes, microphytoplankton were not abundant, so clogging of the smaller mesh size SCOR net did not occur. Consequently, results obtained from comparisons between the two nets were not significantly different (t test, $P > 0.05$) for the macrozooplankton (>250 μm) component of the plankton community. All samples were placed in 125-mL bottles and preserved in sucrose-buffered 4% formalin solution (Haney and Hall 1973). Zooplankton were later counted, identified to genus, and measured with a computerized video measuring system (MacLellan et al. 1993). Body length was measured as described by Koenings et al. (1987). Zooplankton biomass was calculated with species-specific length–weight regressions adapted from Bird and Prairie (1985), Culver et al. (1985), Stemberger and Gilbert (1987), and Yan and Mackie (1987).

For determination of sockeye diet, approximately 25 sockeye stomachs from each trawl sample were analyzed. Only samples collected within 3 h after the onset of darkness were analyzed to avoid bias caused by different digestion rates of prey. Stomach contents were identified and enumerated with the computerized video measuring system. Relative volume of prey types in the stomachs and an index of stomach fullness expressed as a percentage by volume were estimated using a technique modified from Hellawell and Abel (1971).

Lake fertilization

Lake fertilization is a widely used and successful sockeye enhancement technique in British Columbia (Hyatt and Stockner 1985; Stockner and Shortreed 1985). In the mid 1980s Chilko Lake was identified as an excellent candidate for fertilization (Stockner and Shortreed 1983, 1994). Consequently, nitrogen and phosphorus fertilizers were applied to the lake for 6-week periods in 1988 and for 12-week periods in 1990–1993. Details of the application methods and limnological results of the fertilization were reported in Stockner and Shortreed (1991, 1994). In this report, data collected during lake fertilization are identified and reported when pertinent to sockeye growth, zooplankton community composition and biomass, and our estimates of Chilko Lake's sockeye rearing capacity.

Results and discussion

Adult sockeye stock-recruitment relationships

The stock-recruit relationships exhibited by Shuswap Lake sockeye (all stocks combined) and by Adams River sockeye (the Adams River is the major spawning tributary on Shuswap Lake) have been extensively analyzed (Ward and Larkin 1964; Larkin 1971; Collie and Walters 1987; Cass 1989; Collie et al. 1990; Welch and Noakes 1991). On dominant cycles, Adams River sockeye averaged 95% of the total escapement until 1970. Since that time, escapements to other Shuswap Lake tributaries have increased, so that Adams River sockeye now account for about 70% of the total Shuswap Lake escapement in dominant cycles. On the subdominant cycle the Adams River escapement has varied between 75 and 98% during the same period. Adams River escapement during the two nondominant cycles is highly variable, ranging between 2 and 65% (mean 35%) of the total Shuswap Lake escapement. In this study, we use age-4 returns (catch and escapement) and escapement to all spawning grounds of each lake system.

The Pacific Salmon Commission and the Department of Fisheries and Oceans have recently revised their estimates of escapements and returns, resulting in significant changes to some data points since the previous papers were published (W. Saito and T. Whitehouse, Department of Fisheries and Oceans, New Westminster, B.C., personal communication). Using the revised EFS and return data (using brood years from 1948 to 1988) we computed a new S_{MAX} of 2.66 million EFS (83 EFS/ha, Fig. 3). As EFS averages 53% of returning adults in Shuswap Lake, the estimated optimum escapement to Shuswap Lake based on stock-recruit analysis is 5.0 million, 24% higher than the record escapement of 3.98 million in 1990 (Fig. 3). However, we found that the regression of $\log(R/S)$ on S was not significant ($P > 0.05$). We also found considerable variance (as did Collie et al. 1990) with an r^2 of only 6%. With currently available data the Ricker model cannot be differentiated from a linear model.

In Chilko Lake, Collie et al. (1990) again reported a very high variation in the b value (90% CI = ±63%), and a S_{MAX} of 549 000 EFS, with a confidence interval of 337 000 to 1.5 million. As with Shuswap data, we repeated their analysis with the addition of more recent data (excluding returns from fertilized brood years) and computed a S_{MAX} of 457 000 EFS (25/ha), or a total escapement of 896 000 spawners (EFS averaged 51% of total adult escapement, Fig. 3). Although the r^2 value was still low (10.9%), the regression was significant ($P < 0.05$). The first returns from fertilized brood years occurred in 1991 and 1993 and while they were among the highest ever recorded from these escapement levels, they made little difference to the stock-recruit analysis, producing an estimated S_{MAX} of 439 000 EFS when included.

The Ricker b value for Quesnel Lake data is negative, meaning that the curve is nearly linear and S_{MSY} and S_{MAX} cannot be computed (Fig. 3). Since 1977, escapements in dominant brood years have increased from 160 000 to over 900 000 EFS and returns per EFS have declined, indicating some limitation to the productivity of the stock, but there are insufficient data at higher escapement levels to properly estimate the parameters of the Ricker model. Because of this we examined a number of other possible curves. Examination of the residuals showed that a quadratic model fitted the data slightly better than a linear fit (decreasing both the standard error of the residuals and the mean average error by 34 and 30%, respectively). The quadratic curve peaked (equivalent to S_{MAX}) at about 930 000 EFS (36/ha), or a total escapement of 2.0 million (EFS average 46% of total adult escapement, Fig. 3).

Juvenile sockeye populations

In both Quesnel and Shuswap lakes the summer (late July or August) fry densities were linearly correlated with EFS up to densities of about 35 EFS/ha (in both cases $r^2 > 0.87$, $P < 0.05$, Fig. 4). Quesnel and Shuswap lakes exhibited similar EFS to summer fry relationships, indicating that from egg deposition to the following summer, sockeye fry in both lakes had similar survival rates up to EFS numbers of 35/ha (analysis of covariance, neither the slopes nor the intercepts were significantly different from each other, $P < 0.05$). At the two highest observed EFS numbers in Shuswap Lake (50 and 58/ha), summer fry numbers decreased. The lower survival at these densities may be a result of spawning ground or lake rearing limitation.

Fall fry (October to early November) abundance in Shuswap and Quesnel lakes exhibited a strong curvilinear or asymptotic relationship to EFS (Fig. 4). Ricker stock-recruit curves fitted to the data resulted in a S_{MAX} in Shuswap Lake

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Fig. 3. Total adult (4_2) returns to Shuswap, Quesnel, and Chilko lakes produced by effective female spawners. Selected brood years are labelled and the optimum escapements estimated by the photosynthetic rate (PR) model are shown by the two-headed arrows. Ricker stock-recruit curves (solid lines) have been fitted to the data. A quadratic curve was fitted to the Quesnel Lake data.

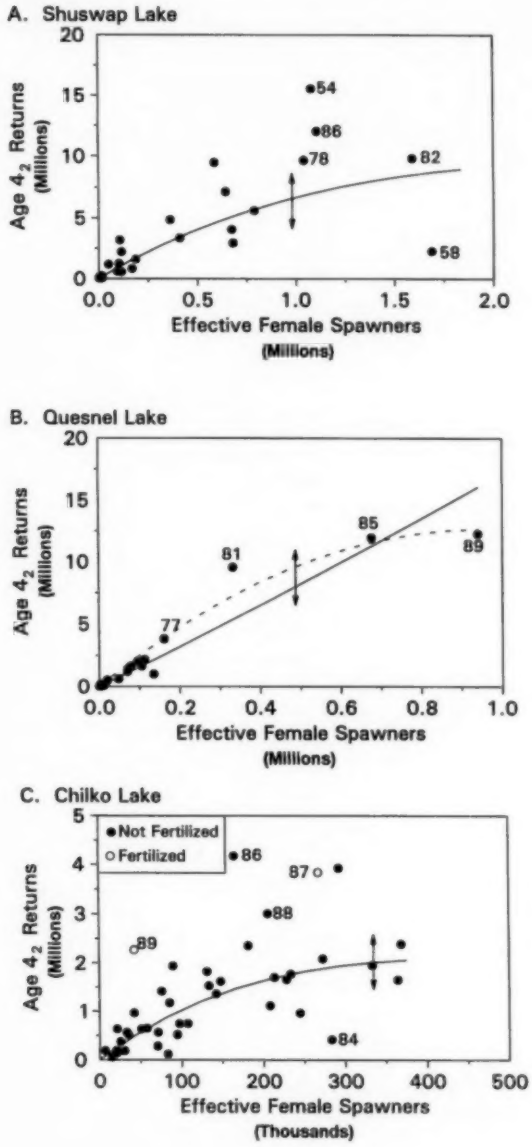
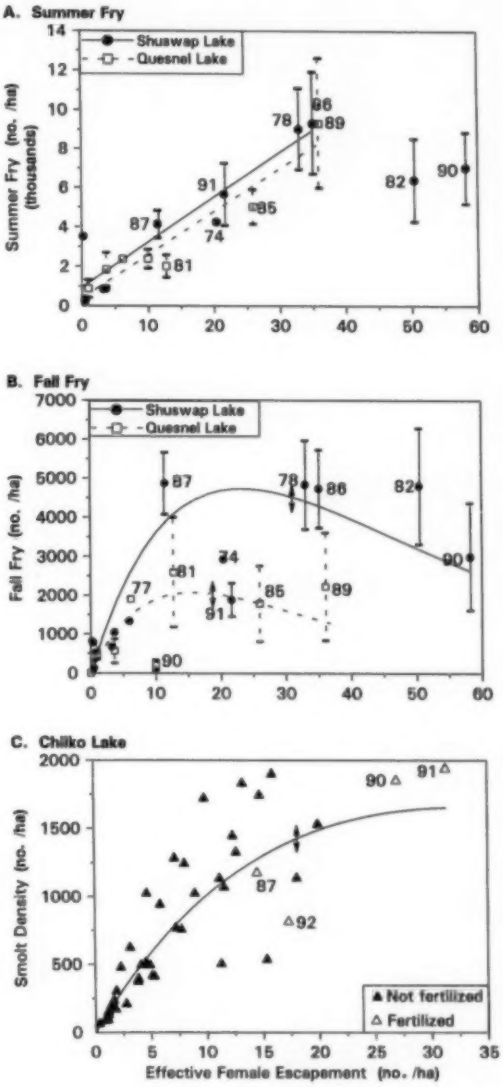


Fig. 4. Summer and autumn fry densities in Shuswap and Quesnel lakes and smolt densities in Chilko Lake produced by effective female spawners. Smolts affected by fertilization in Chilko Lake are indicated by the open triangles. Where possible 95% confidence intervals are shown by a vertical line and horizontal bars. Selected brood years are labelled and the optimum escapements estimated by the photosynthetic rate (PR) model are shown by the two-headed arrows. Ricker stock-recruit curves (solid lines) have been fitted to the data in B and C.



of 23.2 EFS/ha (732 000 EFS, 1.38 million total escapement, $r^2 = 0.51$, $P < 0.05$) and a S_{MAX} in Quesnel Lake of 15.9 EFS/ha (415 000 EFS, 0.90 million total escapement, $r^2 = 0.41$, $P > 0.05$). Although the Ricker model describes these data better than it does the adult stock recruit data, 50% or more of the variation in the relationships was still unexplained.

The 95% confidence intervals of S_{MAX} (based on the regression of $\ln(R/S)$ against S) were still too large (ranging from 9.8 to 41.3 EFS/ha in Shuswap Lake and from 0 to infinity in Quesnel Lake) to be a practical predictive tool. Escapements greater than 25 EFS/ha (total adult escapements of 1.5 million) to

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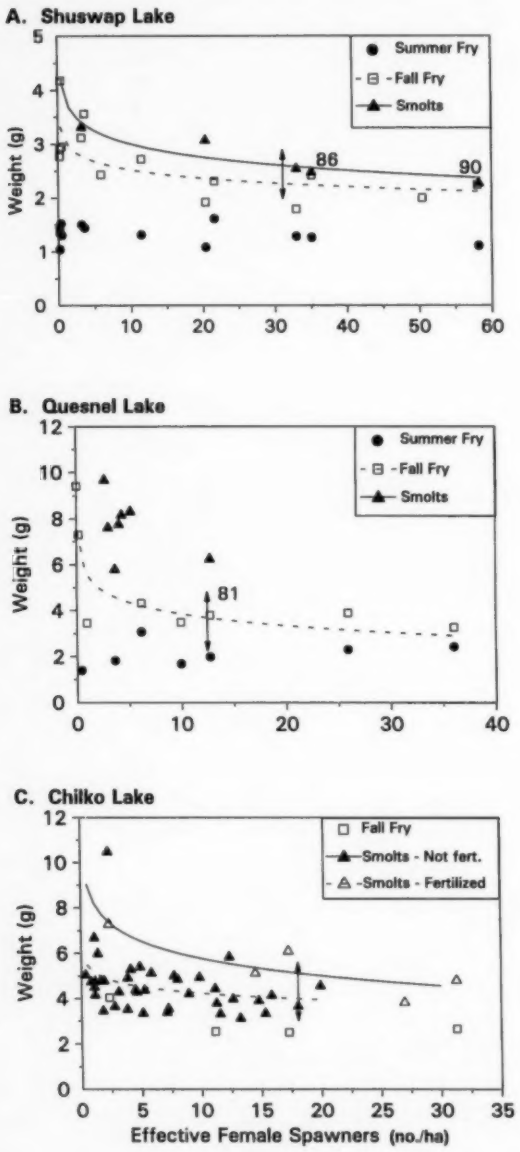
Fig. 5. Size of juvenile sockeye in the summer and fall and as spring smolts in Shuswap, Quesnel, and Chilko lakes. The optimum escapements estimated by the photosynthetic rate (PR) model are shown by the two-headed arrows. Smolts affected by fertilization in Chilko Lake are indicated by the open triangles. Logarithmic curves have been fitted to the fall fry and smolt data.



Shuswap Lake did not produce any more fall fry, peaking at 4900 fry/ha. Similarly escapements to Quesnel Lake of 15 EFS/ha (total adult escapements of 0.8 million) also did not produce any more fry, peaking at 2600 fry/ha. These observed escapements agree closely with the Ricker S_{MAX} calculated for the two lakes.

In Chilko Lake production of smolts per EFS decreased only slightly with increasing EFS escapement. While the Ricker fit was significant ($r^2 = 0.29$, $P < 0.05$), a linear fit was better ($r^2 = 0.64$, $P < 0.05$, Fig. 4). The Ricker analysis resulted in a S_{MAX} of 31.1 EFS/ha (575 000 EFS, 1.06 million adults). This is beyond the highest observed EFS escapement and had 95% confidence intervals of 27.0–68.3 EFS/ha. However, interpretation of the effects of spawning density on smolt production was complicated by lake fertilization. Juvenile sockeye from two of the highest escapements (26 and 31 EFS/ha) were from fertilization years. Since a well-documented effect of lake fertilization is increased fry to smolt survival (Hyatt and Stockner 1985; Koenings and Burkett 1987), these 2 brood years may have produced fewer smolts if the lake had not been fertilized. As a result, S_{MAX} would have been smaller. In any case, even with fertilization, no more smolts were produced at densities of 25–31 EFS/ha than at densities of 15 EFS/ha (i.e., maximum observed smolt output was reached at total adult escapements of 0.5 million).

Other lakes have exhibited EFS to fall fry relationships similar to those found in our study. Babine Lake is a large (491 km^2 in area) sockeye producer in the Skeena River system and is the only British Columbia sockeye lake for which direct estimates of fry recruitment are available (McDonald and Hume 1984; MacDonald et al. 1987). Densities of fry at the time of emergence from the gravel ranged from 1000 to 10 300 fry/ha (approximately 13 to 29 EFS/ha) and there was a linear relationship between emergent fry numbers and subsequent smolt production (i.e., no density-dependent survival). At this range of densities fry to smolt survival averaged 35%. In Leisure Lake, a much smaller (1.1 km^2) lake in Alaska, Koenings and Burkett (1987) found that smolt numbers did not increase after spring fry numbers exceeded 10 000/ha. At densities >10 000 fry/ha (approximately 19 EFS/ha) smolt numbers did not increase further, with smolt numbers declining at the highest fry densities. These results are very similar to the curvilinear relationship we found between EFS and subsequent summer and fall fry numbers in Shuswap and Quesnel lakes (Fig. 4).

Juvenile sockeye size

Summer fry size in Quesnel and Shuswap lakes did not vary with spawner density ($P > 0.05$, Fig. 5). Summer fry averaged 2.1 g (range 1.3–3.1 g) in Quesnel Lake and 1.3 g (range 1.1–1.6 g) in Shuswap Lake. Possible causes for these size differences are discussed in a later section and are most likely related to differences in thermal regimes between the two lakes.

Fall fry and smolt size in all three lakes declined rapidly as EFS density increased to about 10 EFS/ha. At escapements >10 EFS/ha fall fry size did not significantly decline in either Shuswap or Quesnel lakes (Fig. 5). Chilko Lake smolt size also showed little density-dependent effects at escapements >10 EFS/ha, but results at the highest densities in Chilko Lake were confounded by fertilization. Smolts from fertilized years tended to be larger than smolts from comparable unfertilized years, and the highest EFS densities in Chilko Lake occurred only during fertilized years (Fig. 5). In an Alaskan lake, Koenings and Burkett (1987) also found a decreasing change in growth rates at the highest stocking densities. In our study lakes, brood years when both fall fry and smolt sizes are available are limited, but available data do show that overwintering

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growth can exceed 2 g in low-density brood years (e.g., 1981 in Quesnel Lake). In high-density brood years overwintering growth can be negligible (1986 and 1990 in Shuswap Lake, Fig. 5).

Fall fry and returning adults

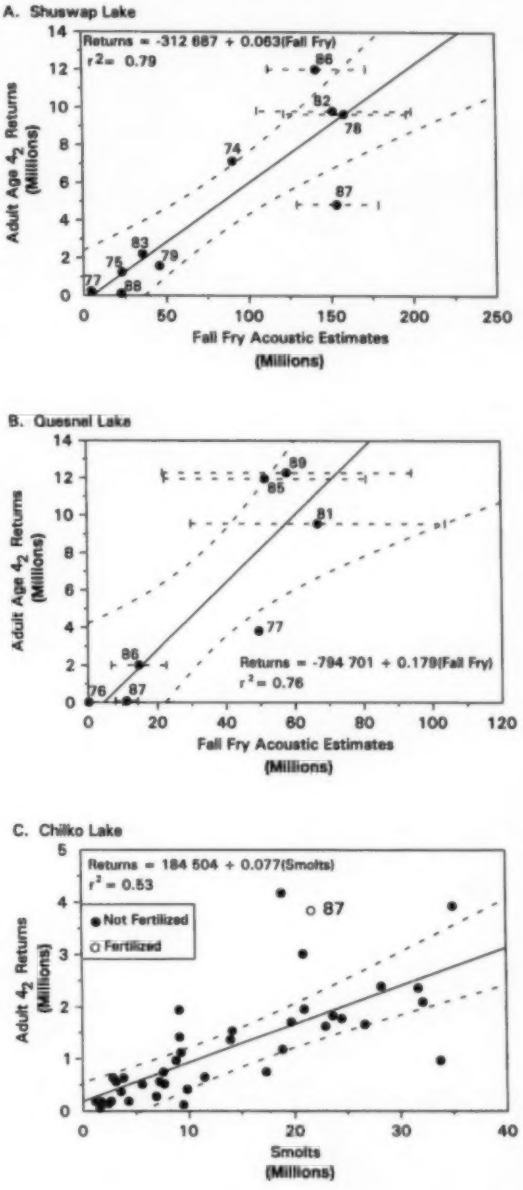
Unlike the lake resident life history, there were no obvious density-dependent effects on marine survival for any of our study lakes. In all three lakes there were highly significant linear relationships between numbers of fall fry or smolts and subsequent adult returns (Fig. 6). Fall fry to adult survival averaged 5.6% for Shuswap Lake and 14.8% for Quesnel Lake. At Chilko Lake smolt to adult survival averaged 9.1%. These survival rates were not significantly related to density but were significantly different from each other (least significant difference test on angular transformed data, $P < 0.05$, Sokal and Rohlf 1981). Fall fry in Quesnel Lake averaged about 4 g at higher densities, about twice the 2-g size of Shuswap Lake fall fry and the same size as Chilko Lake smolts. The limited smolt data available for our lakes indicate that at equivalent EFS densities Shuswap Lake produced the smallest smolts and Quesnel the largest. This may indicate size-dependent smolt to adult survival as seen in other sockeye populations (Hyatt and Stockner 1985; Koenings et al. 1993), where smolt to adult survival rates were strongly size dependent over the size ranges seen in this study. However, different winter and early spring growth rates because of different EFS densities and environmental conditions make it inappropriate to estimate smolt size from fall fry size. There are few smolt size data for any Fraser system lake except Chilko Lake, where considerable smolt data are available. Chilko smolt data do not exhibit a relationship between size and survival from smolts to adults between brood years. However, Henderson and Cass (1991) did find a relationship within brood years at Chilko Lake. They speculated that factors such as run timing, river conditions, or total system smolt abundance may play a larger role than smolt size in determining survival rates between brood years in the Fraser River system.

Macrozooplankton and sockeye planktivory

The role of selective planktivory in structuring zooplankton community composition has been well documented (see Northcote 1988 for review). Intense salmonoid planktivory has been linked with a reduced abundance of large-bodied zooplankton in a number of lakes (Brooks and Dodson 1965; Brooks 1969; Stenson 1972, 1976; Kerfoot 1975; Kerfoot and Sih 1987; Goodlad et al. 1974; Kyle et al. 1988). While intense grazing pressure may have a large effect on zooplankton community composition, the effect on total macrozooplankton biomass may be much less pronounced (Kyle et al. 1988, our Table 1). Principal diet items of juvenile sockeye differed among our study lakes.To document depletion of their forage base, we needed to first determine preferred diet items by stomach content analysis. For example, macrozooplankton biomass was not substantially affected by high fish density in Shuswap Lake, where sockeye grazed almost entirely on *Daphnia* spp. (Table 1). However, *Daphnia* biomass declined significantly with increased grazing pressure (Fig. 7).

In Shuswap and Quesnel lakes, the copepods *Leptodiaptomus* and *Diacyclops* spp. constituted 45–80% of macrozooplankton biomass. Daphnids were the dominant cladocerans (30–50%)

Fig. 6. Relationship between fall fry and subsequent adult (4_2) returns. The broken line indicates the 95% confidence intervals on the regression lines and where possible 95% confidence intervals on the data points are shown by a horizontal line and vertical bars. Selected brood years are labelled.



in these lakes, except during the fall of dominant years, when they declined to 13–30%. Significant negative correlations occurred between EFS and *Daphnia* biomass in both Shuswap ($r^2 = 0.96$, $P < 0.01$) and Quesnel ($r^2 = 0.93$, $P < 0.05$) lakes (Fig. 7). In both lakes average *Daphnia* biomass decreased by approximately 60% in dominant brood years, declining from an average of 522 mg/m^2 (nondominant) to 224 mg/m^2 in

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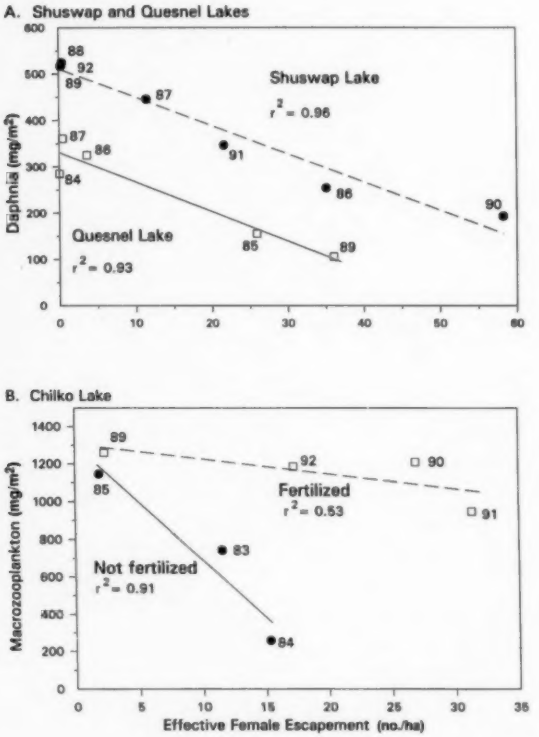
Table 1. Seasonal means of salient limnological variables from the study lakes.

	Mean epilimnetic temp. (°C)	Epilimnetic depth (m)	EZD (m)	TP (μg/L)	Nitrate (μg/L)	CHL (μg/L)	Daily PR (mg C/m²)	*Daphnia* biomass (mg/m²)	Macrozooplankton biomass (mg/m²)
Chilko									
Unfertilized	7.5	21.4	14.8	2.3	12.0	0.68	79	4.7	715
	(0.3)	(0.5)	(5.1)	(0.6)	(3.9)	(0.24)	(1.0)	(8.4)	(513)
Fertilized	9.3	17.6	20.5	4.1	12.7	0.88	103	152	1150
	(1.2)	(2.90)	(1.9)	(0.3)	(3.4)	(0.34)	(30)	(192)	(139)
Shuswap	14.9	10.0	12.3	5.1	18.4	1.81	171	400	1005
	(0.5)	(1.1)	(0.5)	(1.1)	(5.8)	(0.31)	(23)	(104)	(141)
Quesnel	12.4	12.2	15.1	2.7	69.3	1.03	102	247	894
	(0.4)	(1.5)	(1.0)	(0.4)	(8.8)	(0.07)	(11)	(99)	(278)

Note: Values in parentheses are two standard errors. Chilko Lake unfertilized averages were calculated for the years 1984–1986 ($n = 3$) and fertilized averages for 1990–1993 ($n = 4$). Quesnel Lake averages are for 1985–1989 and 1993 ($n = 6$). Shuswap Lake averages are for 1987–1993 ($n = 7$).

Fig. 7. Mean seasonal (summer to fall) *Daphnia* biomass in Shuswap and Quesnel lakes, and mean seasonal macrozooplankton in Chilko Lake. Brood years (1 year prior to sampling) are labelled.



Shuswap Lake and from 323 to 131 mg/m² in Quesnel Lake. In Shuswap Lake *Daphnia* biomass was significantly higher at equivalent fry densities than in Quesnel Lake (covariance analysis; equal slopes, $F = 0.84$, $P > 0.05$; different intercepts, $F = 55.12$, $P < 0.01$; Sokal and Rohlf 1981). *Daphnia* spp. comprised 90% of total stomach volume in Quesnel and Shuswap lakes, except in the fall of dominant brood years. At those times sockeye diet consisted of the smaller cladocerans *Eubosmina* spp., the copepods *Diacyclops* and *Leptodiaptomus* spp., and small quantities of *Daphnia* spp. Stomach fullness averaged >90% when *Daphnia* spp. were the major food item in Shuswap and Quesnel lakes and <50% when *Daphnia* spp. were depleted and sockeye were forced to switch prey.

Before fertilization in Chilko Lake, macrozooplankton community biomass was dominated by the small cladocerans *Eubosmina* spp. (10–50%) and by the copepods *Diacyclops* (20–30%) and *Leptodiaptomus* spp. (40–60%). These three genera comprised 100% of sockeye stomach contents. *Daphnia* spp. were not found in the stomachs examined and they comprised <1% of total macrozooplankton biomass (Table 1). Prior to fertilization, we found no change in species composition with increasing EFS densities, but there were large changes in biomass. Macrozooplankton biomass declined rapidly with increasing sockeye density, from 1145 mg/m² at 2 EFS/ha to 257 mg/m² at 15 EFS/ha (Fig. 7). In contrast, during fertilization, increasing EFS density had little effect on macrozooplankton biomass. Even with density at a historic maximum of 31 EFS/ha, mean seasonal macrozooplankton biomass was >900 mg/m² (Fig. 7). In spite of high sockeye densities during 3 of the 4 fertilized years there was a large increase in *Daphnia* biomass during fertilization. After averaging 4.7 mg/m² prior to fertilization, *Daphnia* biomass averaged 152 mg/m² during fertilized years (Table 1). As a proportion of macrozooplankton biomass, *Daphnia* spp. increased from <1 to 13% during fertilized years. This occurred even though daphnids became a major component (up to 75% of stomach volume) of sockeye diet in some fertilized years. Stomach fullness also increased from an average of <25%, containing only *Eubosmina*, *Diacyclops*, and *Leptodiaptomus* spp., in unfertilized years, to >75% full, containing primarily *Daphnia* and *Eubosmina* spp.

Lake physics

Thermal regimes varied considerably between the three lakes in this study. With its milder climate and calmer conditions, Shuswap Lake is stratified from early May to November and has epilimnetic temperatures >20°C from early July until mid September. It has a very strong, stable, and shallow (10 m) seasonal thermocline (Table 1). Because of the warm epilimnion, juvenile sockeye are restricted in their use of this productive area during this period (Levy 1989; J.M.B. Hume, unpublished data). Quesnel Lake also stratifies strongly, but to

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a lesser degree than does Shuswap Lake. Although stratification is stable from June to October, epilimnetic temperatures rarely exceed 17°C, and the average depth of the epilimnion is 12.2 m. On occasion the Quesnel Lake epilimnion is not utilized by juvenile sockeye (Levy et al. 1991), but for much of the summer and fall grazing sockeye can use the whole water column. In Shuswap Lake July–September epilimnetic (<10 m) *Daphnia* biomass averaged 13 times greater than biomass below the thermocline (10–30 m), while in Quesnel Lake epilimnetic *Daphnia* biomass averaged only twice as great as deeper biomass. We suggest that because of its stronger stratification and warmer epilimnion, Shuswap Lake provides a thermal refuge for zooplankton, resulting in a larger proportion of the zooplankton community being unavailable to grazing sockeye. As a result of this refuge, Shuswap Lake maintains higher *Daphnia* numbers than Quesnel Lake but produces smaller fall fry at similar fish densities. Chilko Lake's high elevation and exposure to frequent strong winds result in a dynamic thermal structure and a cool epilimnion, with summer epilimnetic temperatures seldom exceeding 14°C. Thermal stratification does develop (July–October) on Chilko Lake, but epilimnion depths are variable, ranging from 10 to 50 m, and the entire water column is available to foraging sockeye. Our study lakes were all clear, with average EZDs ranging from 12.3 m in Shuswap Lake to 20.5 m in Chilko Lake (Table 1).

Trophic status and lake productivity

We found that the study lakes are oligotrophic, with spring TP concentrations <10 µg/L. Nevertheless, they cover a wide range of oligotrophy, with Chilko being ultraoligotrophic, Quesnel slightly more productive, and Shuswap approaching mesotrophy. Phosphorus availability is the primary factor limiting productivity in all three lakes, but there are substantial differences between lakes. Seasonal average TP was 2.3 µg/L in unfertilized Chilko Lake, 2.7 µg/L in Quesnel Lake, and 5.1 µg/L in Shuswap Lake. During summer in Shuswap Lake and at times in Chilko Lake, a complex co-limitation of nitrogen and phosphorus occurs (Stockner and Shortreed 1994; K.S. Shortreed, unpublished data). Quesnel Lake has an abundant nitrogen supply, so phosphorus is the major limiting nutrient throughout the season (Table 1). Average phytoplankton biomass (as CHL) was highest (1.81 µg/L) in Shuswap Lake and lowest (0.68 µg/L) in unfertilized Chilko Lake. Average CHL was 1.03 µg/L in Quesnel Lake and 0.88 µg/L in fertilized Chilko Lake. PR also reflected the differing productivities of the three lakes. Average seasonal PR was 102 mg $C \cdot m^{-2} \cdot d^{-1}$ in Quesnel Lake and 171 mg $C \cdot m^{-2} \cdot d^{-1}$ in Shuswap Lake. Chilko Lake PR averaged 79 mg $C \cdot m^{-2} \cdot d^{-1}$ in unfertilized years and 103 mg $C \cdot m^{-2} \cdot d^{-1}$ in fertilized years (Table 1).

Predictive models for lake rearing capacity

A predictive model that reliably characterizes a lake's rearing capacity for juvenile sockeye would be a valuable tool in determining optimum adult sockeye escapements. To date such models have not been available for British Columbia lakes. A model was developed by Alaskan limnologists (Koenings and Burkett 1987) and is being successfully used for management and enhancement of some Alaskan sockeye stocks. This euphotic volume (EV) model provides Alaskan fisheries managers with a method for setting escapement goals for lakes that incorporates actual lake productivities. At present in Alaska, the EV model together with freshwater and ocean survival data (Koenings et al. 1993) enables Alaskan managers to set escapement goals that incorporate both lake productivity and optimum smolt size for maximizing adult production. The EV model is based on the premise that a lake's carbon production (determined from PR measurements) is correlated to rearing capacity. Several studies have correlated various measures of lake productivity with fish yield (Oglesby 1977; Liang et al. 1981; Mills and Schiavone 1982; Fee et al. 1985). One disadvantage of determining PR is that it is a more difficult measurement to obtain than most limnological variables. Koenings and Burkett (1987) found that in their study lakes, PR (seasonal average values expressed as mg $C \cdot m^{-2} \cdot d^{-1}$) was strongly and positively correlated to seasonal average EZD ($PR = 13.9(EZD) - 17.6$, $r^2 = 0.81$, $df = 11$). This occurred because widely varying levels of glacial turbidity resulted in their lakes having a relatively narrow range of nutrient loadings and a wide range in clarity. Because EZD is relatively easy to measure they used it as a surrogate for PR. They then multiplied EZD by lake surface area to yield the total volume of a lake's euphotic zone. One EV unit was defined as 10^6 m^3 of euphotic zone. In a series of experimental manipulations of fry numbers, Koenings and Burkett (1987) found that maximum numbers of smolts (23 000/EV) were produced at spawning escapements of 400–450 adults/EV. Further increasing escapements resulted in increased freshwater mortality, decreased growth, and decreased marine survival. This resulted in production of the same or fewer numbers of smaller smolts, lower marine survival, and lower adult returns. Adult sockeye productionwas maximal at an average spawning density of 425 adults/EV, which produced 23 000/EV of 4- to 5-g smolts (Koenings and Burkett 1987; Koenings et al. 1993).

A similar model would be a useful tool in British Columbia sockeye lakes, but in Fraser River system lakes, as with most regions in North America, PR and EZD are negatively correlated (Fig. 8). This occurs because Fraser system lakes have a narrower range of water clarity and a wider range of nutrient loading than found in the Alaskan lakes (Koenings and Burkett 1987). While EZD is a suitable surrogate for PR in the Alaskan lakes studied, this is not true for Fraser system lakes.

Since we routinely measure PR in our study lakes, we modified the Alaskan EV model to directly use PR rather than its surrogate EZD. We did this by replotting the Alaskan PR and EZD data with EZD as the dependent variable. The resulting equation was

$$EZD = 0.0583 \times PR + 3.25$$

where EZD is the seasonal average euphotic zone depth (m) and PR is the seasonal average PR (mg $C \cdot m^{-2} \cdot d^{-1}$). The original calculation of EV units was

$$\text{EV units} = \frac{\text{EZD (lake area)}}{10^6}$$

where lake area is the lake surface area (m^2). We then modified the calculation of EV units to use PR instead of EZD. Our modified equation was

$$\text{PR units} = \frac{(0.0583 \times PR + 3.25)\ \text{(lake area)}}{10^6}$$

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Table 2. Predicted optimum escapements (S_{MAX}) of adult sockeye with predicted and observed smolt output from our study lakes.

Lake	Lake area (km^2)	PR units	Predicted optimum escapement (millions)			Maximum smolt output (millions)	
			From PR model	From adult SR model	From juveniles[a]	Predicted from PR	Observed
Chilko							
Unfertilized	185	1447	0.62	0.90	1.06	33	34
			(1436–1458)	(0.57–0.66)		(0.81–2.45)	
Fertilized	185	1706	0.72			39	
		(1382–2033)	(0.55–0.91)				
Quesnel	270	2494	1.06	0.93[b]	0.90	57	47[c]
		(2314–2674)	(0.93–1.20)	(0–infinity)			
Shuswap	330	4363	1.85	4.7	1.38	100	107[c]
		(3912–4814)	(1.56–2.17)	(0.54–2.59)			

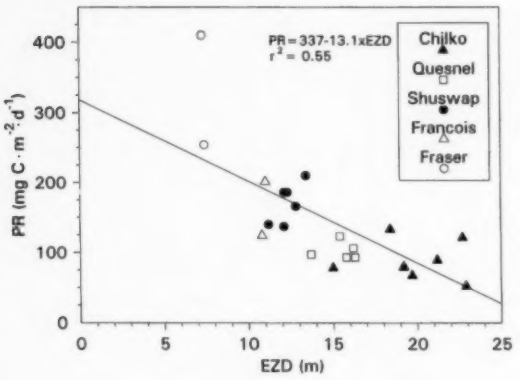
Note: Estimated range or 95% confidence limits are given in parentheses. See text for calculation methods. PR, photosynthetic rate model; SR, adult spawner recruit model.

[a]Smolts in Chilko Lake, fall fry in Quesnel and Shuswap lakes.

[b]Quadratic fit.

[c]Quesnel and Shuswap smolt numbers estimated from maximum fall fry numbers (2600 and 4900/ha, respectively) and a fall–spring mortality of 7%/month (Koenings and Burkett 1987; Hume et al. 1994).

Fig. 8. Relationship between seasonal averages of photosynthetic rate (PR) and euphotic zone depth (EZD) in Fraser River lakes.



We then entered our PR data into the equation and could calculate optimum escapements and smolt outputs for Fraser system lakes. For clarity, we call the revised model the PR model.

As stated previously, Koenings and Burkett (1987) found that in the lakes they studied maximum smolt output was 23 000/EV unit and that maximum subsequent adult returns were achieved at escapements averaging 425/EV unit. We calculated optimum escapements and maximum smolt output for our study lakes and for a number of other B.C. sockeye lakes using the factors of 23 000 smolts/PR unit and 425 spawners/PR unit (Tables 2, 3). Predicted optimum total adult escapements (equivalent to S_{MAX}) for Chilko, Quesnel, and Shuswap lakes were 0.62, 1.06, and 1.85 million (18.1, 18.7, and 31.1 EFS/ha), respectively. While a true 95% confidence interval cannot be determined, a range can be estimated on the basis of the standard error of the annual variation in the PR data and the range of optimum spawning densities found in the Alaskan lakes studied. This was about 6% of the estimated optimum for each lake and overlapped the confidence range for S_{MAX} based on juvenile data in Quesnel and Shuswap lakes, but not in Chilko Lake. However, because of fertilization and limited data points at higher escapements, there is considerable uncertainty about the optimum escapements predicted by Chilko smolt data. It is worth noting that the PR model indicates that optimum escapements to Chilko are 15 EFS/ha, and existing data indicate that no more smolts were produced at densities of 25–30 EFS/ha when the lake was fertilized than were produced at densities of 15 EFS/ha when the lake was not fertilized (Table 2, Fig. 4).

If a lake's PR units are an effective indicator of a lake's rearing capacity, then the biomass of juvenile sockeye a lake can produce should be correlated to its PR units. To test this we regressed maximum observed fall fry or smolt biomass from our study lakes against PR units. Since fall fry biomass was not available for Chilko Lake and smolt biomass was not available for Quesnel and Shuswap lakes, we assumed that smolt biomass was equivalent to fall fry biomass (i.e., losses owing to mortality were offset by late fall and spring growth of surviving juveniles). We also utilized data from two additional lakes that we believe are producing maximum biomass. Fraser Lake is a relatively small (53 km^2) Fraser system lake for which we have (unpublished) fall fry data from a high-density brood year. Babine Lake was described previously and data on smolt numbers and size are available (MacDonald et al. 1987). Despite the small sample size and assumptions about growth and survival (i.e., we had to utilize both fall fry and smolt data), the data are highly correlated ($P < 0.01$, $r = 0.93$, Fig. 9). As a further test of the utility of PR units in determining lake rearing capacity, we normalized both fall fry biomass and EFS to PR units for Quesnel and Shuswap lakes. Although the data sets and fitted Ricker curves are very different when normalized to lake area (Fig. 4), they are quite similar when normalized to PR units, particularly maximum fall fry biomass (Fig.10).

Adult sockeye stock-recruitment relationships for Chilko, Quesnel, and Shuswap lakes are of limited use in predicting

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Table 3. Predicted optimum escapements and smolt output from additional B.C. lakes.

Lake	Area (km^2)	PR units	Predictions from PR model	
			Optimum escapement	Maximum smolt output
Alastair	6.7	101	4.3×10^4	2.3×10^6
Babine[a]	475	5546	2.4×10^6	1.3×10^8
Bear	19	255	1.1×10^5	5.8×10^6
Francois	247	3151	1.34×10^6	7.2×10^7
Fraser	53	1216	5.2×10^5	2.8×10^7
Fred Wright	3.9	40	1.7×10^4	9.1×10^5
Johanson	1.4	10	4.2×10^3	2.3×10^5
Kitlope	12	71	3.0×10^4	1.6×10^6
Kitsumkalum	18	95	4.1×10^4	2.2×10^6
Lakelse	13	134	5.7×10^4	3.1×10^6
Meziadin	36	438	1.9×10^5	1.0×10^7
Morice	96	796	3.4×10^5	1.8×10^7
Nimpkish	37	254	1.1×10^5	5.8×10^6
Owikeno	91	589	2.5×10^5	1.4×10^7
Swan	18	176	7.5×10^4	4.0×10^6
Woss	13	110	4.7×10^4	2.5×10^6

Note: These are B.C. sockeye lakes in which optimum escapements have not been determined and in most cases they are recruitment limited. PR data on these lakes are from Stockner and Shortreed (1979), Stockner et al. (1980), Shortreed and Stockner (1981), and K.S. Shortreed (unpublished data).

[a]Most spawning in Babine Lake occurs in spawning channels where egg to fry survival is higher than in natural streams (MacDonald and Hume 1984). Consequently, the PR model overestimates the optimum escapement to Babine Lake.

Fig. 9. Maximum observed fall fry or smolt biomass in British Columbia lakes that are at or near optimum escapement. Data for Fraser Lake are the authors' and those for Babine Lake are from MacDonald et al. (1987).

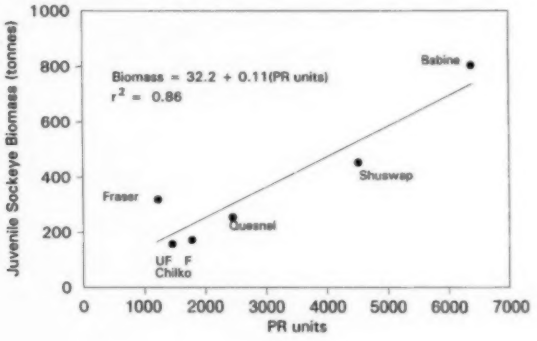
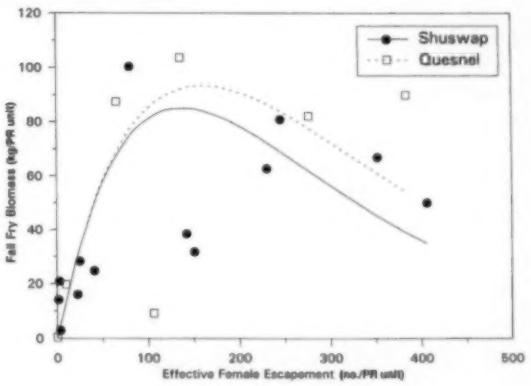


Fig. 10. Relationship between fall fry and effective female spawners in Shuswap and Quesnel lakes, normalized by photosynthetic rate (PR) units. Ricker stock-recruit curves have been fitted to the data.



optimum escapements because of the considerable scatter and because of the paucity of escapements beyond S_{MAX} (Fig. 3). With current data, juvenile sockeye (fall fry or smolt) numbers from these lakes provide a better estimate of escapements that maximize smolt production (Fig. 4). Current data indicate that fall fry or smolt numbers do not increase beyond total adult escapements of 1.4 million to Shuswap, 0.9 million to Quesnel, and 0.5 million to Chilko lakes. Predicted optimum escapements generated by the PR model agree reasonably well with those that produced maximum fry numbers (Fig. 4, Table 2).

In the Alaskan lakes studied, the EV model is an effective tool in predicting a lake's rearing capacity. We suggest that our PR model, which is a simple modification of the Alaskan EV model, will be a useful tool in B.C. lakes. When sufficient data are available, relationships between EFS and fall fry or smolt numbers provide a direct estimate of the optimum escapement to a lake. In addition, they are useful in predicting adult returns. Reliable PR data can be obtained in monthly surveys carried out from spring to fall for 1–2 years. There are many lakes in British Columbia for which we have PR data and for which sockeye production is currently recruitment limited (Table 3). Enhancement through catch management or other techniques is being considered or attempted on a number of these lakes. The PR model provides an estimate of the rearing capacity of these lakes that is based on actual lake

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productivity and that enables a manager to determine the amount of stock rebuilding that is desirable (Table 3).

Clearly, however, other factors can affect a lake's ability to produce sockeye and must be considered when setting escapement goals. Thermal regime, plankton community structure or productivity, and predator–competitor populations (Burgner 1987) all affect rearing capacity. Further, implicit in the use of the PR model to predict optimum escapements is the assumption that the relationship between spawners and fry recruitment is similar in B.C. and Alaskan lakes and that there is no spawning ground limitation. Another factor to be considered is how our estimates of optimum escapements relate to the cycles exhibited by some Fraser system sockeye stocks. Can the lakes sustain continuous optimum escapements or are lower nondominant returns necessary within each 4-year cycle? Further research is needed to better understand these factors and perhaps incorporate them together with zooplankton productivity in an improved version of the PR model. However, we conclude that in its current simple form the PR model will be a useful tool in calculating optimum escapements in British Columbia sockeye nursery lakes.

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Measuring the bioenergetic cost of fish activity in situ using a globally dispersed radiotracer (^{137}Cs)

David J. Rowan and Joseph B. Rasmussen

Abstract: The energetic cost of activity is an important component of the bioenergetic budget of fish, yet this parameter has rarely been quantified for wild populations. Using a ^{137}Cs mass balance approach, we estimated the annual bioenergetic budgets for individual age-classes of 19 species of North American freshwater fish. Immature fish have low activity-related metabolic costs that agree with estimates based on swimming speed or integer multipliers. Mature fish have 2- to 4-fold higher activity than immature fish and 2- to 4-fold higher activity than estimates based on swimming speed or integer multipliers. The higher activity in mature fish may be due to reproductive efforts. Underestimation of activity in conventional bioenergetics models leads to underestimation of consumption rates. Thus, our in situ and age-specific estimates of activity costs provide a means to improve bioenergetic predictions. Although our analysis was done on an annual basis, it is possible to use the ^{137}Cs technique over shorter intervals (weeks). The ^{137}Cs method has general applicability to aquatic systems because ^{137}Cs is globally dispersed and can be accurately measured in all aquatic organisms using gamma spectrometry.

Résumé : Le coût énergétique de l'activité du poisson est une composante importante de son bilan bioénergétique; or, ce paramètre a rarement été mesuré dans les populations naturelles. Au moyen de la méthode du bilan du ^{137}Cs, nous avons estimé les bilans bioénergétiques annuels des diverses classes d'âge de 19 espèces de poisson d'eau douce d'Amérique du Nord. Le poisson immature présente de faible coûts métaboliques reliés à son activité, qui correspondent aux estimations basées sur des multiples de sa vitesse de nage ou de nombres entiers. Le poisson mature est de 2 à 4 fois plus actif que le poisson immature, et de 2 à 4 fois plus actif qu'il est estimé d'après des multiples de sa vitesse de nage ou de nombres entiers. La plus grande activité du poisson mature pourrait tenir à ses efforts de reproduction. Sous-estimer l'activité dans les bilans bioénergétiques classiques entraîne une sous-estimation des taux de consommation. Nos estimations in situ et par classe d'âge des coûts de l'activité du poisson constituent donc un moyen d'améliorer les prévisions bioénergétiques. Même si notre analyse a été effectuée sur une base annuelle, il est possible d'appliquer la technique du ^{137}Cs à des périodes plus courtes (semaines). La méthode du ^{137}Cs est applicable en général aux systèmes aquatiques, parce que le ^{137}Cs est dispersé partout dans le monde et peut être mesuré avec précision dans tous les organismes aquatiques par spectrométrie gamma. [Traduit par la Rédaction]

Introduction

Bioenergetics modelling is becoming an important tool in fishery research, where it is being used to set stocking levels and catch quotas on the basis of available forage (Stewart et al. 1981; Hartman and Margraf 1992). One of the main criticisms of this approach is the paucity of field measurements of the metabolic cost of activity (Boisclair and Leggett 1989; Ney 1993; Boisclair and Sirois 1993). Because activity is a major component of fish bioenergetic budgets (up to 40% of consumption) that varies considerably among populations (Boisclair and Leggett 1989), modelling results could be greatly strengthened if a more accurate and in situ method of estimating activity were available.

Most bioenergetic models take the form of

$$(1) \quad C = G + MA + S + F + U$$

where C is the consumption rate (g food·g body mass^{-1}·d^{-1}), G is the specific growth rate (somatic and gonadal) (g·g^{-1}·d^{-1}), M is the resting metabolic rate (g·g^{-1}·d^{-1}), A is activity (dimensionless), S is specific dynamic action (g·g^{-1}·d^{-1}), F is egestion (g·g^{-1}·d^{-1}), and U is excretion (g·g^{-1}·d^{-1}) (Winberg 1956; Kitchell et al. 1977; Hewett and Johnson 1992). Typically, resting metabolism, activity, specific dynamic action, egestion, and excretion are estimated from generic parameters, either growth or consumption are measured, and these are used to solve for the remaining term.

Activity, a multiplication factor of resting metabolism that includes metabolic costs associated with swimming as well as other biochemical processes exclusive of specific dynamic action (which is treated separately), is usually estimated in one of two ways. First, activity can be computed by assuming an average swimming speed and then using laboratory-derived empirical relationships (available for few species) between metabolic costs and swimming speed, temperature, and body size (Hewett and Johnson 1992). There are great difficulties

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D.J. Rowan.[1] Environmental Research, Chalk River Laboratories, Chalk River, ON K0J 1J0, Canada.
J.B. Rasmussen. Department of Biology, McGill University, 1205, avenue Docteur Penfield, Montreal, QC H3A 1B1, Canada.

[1] Author to whom all correspondence should be addressed at the following address: Department of Radiological Health Sciences, Colorado State University, Fort Collins, CO 80523-1673, U.S.A.

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with this approach because in situ swimming speeds can only be obtained for a small number of large fish using telemetry (Diana 1980) or by observations of small fish with underwater video cameras (Boisclair and Sirois 1993; Boisclair 1992) and, in both instances, for restricted periods of observation. Another problem with this approach is that small, spatially restricted motions that may account for the bulk of swimming-related activity for many fish (e.g., esocids and centrarchids) are undetectable by telemetry and difficult to discern from video recordings (Ney 1993). If swimming speeds and the metabolic cost – swimming speed relationship is lacking (as it is for most species), activity is simply assumed to be an integer multiplier of resting metabolism and is set between 1 and 2 (Winberg 1956; Kitchell et al. 1977; Hewett and Johnson 1992).

Alternative means of estimating activity include measuring both consumption and growth (Boisclair and Leggett 1989; Post 1990; Madon and Culver 1993) and then solving for the remaining unknown (activity) or through heart rate (Priede and Young 1977; Lucas et al. 1991) or electromyogram (Rodgers et al. 1981; Weatherly et al. 1982) telemetry. The former is extremely laborious, impractical on an ecosystem level, and difficult for the large salmonids or centrarchids that have been the focus of most bioenergetic management strategies. Telemetry of heart rate or oxygen consumption has the advantage of directly measuring field metabolic costs (Holliday et al. 1974; Weatherly et al. 1980), including activity, but is limited to small numbers of large fish over restricted periods of observation. Both of these methods are of use for validating model assumptions and addressing ecological questions but not for making routine bioenergetic estimates of consumption or growth. However, these studies have shown that activity costs can be as high as 4-fold resting metabolism (Boisclair and Leggett 1989; Post 1990; Madon and Culver 1993) and variable among systems (Boisclair and Leggett 1989; Madon and Culver 1993); 4-fold errors in activity can easily translate into 2-fold errors in consumption estimates.

To estimate age-specific activity costs for wild populations, a more practical method of estimating consumption rates than measurement of gut contents and gut clearance rates (Eggers 1977; Elliott and Persson 1978) is required. We propose such a method based on the mass balances of growth and ^{137}Cs. The strength of this approach is that consumption is measured independent of metabolism, and then the difference can be used to solve for activity. Instead of modelling the nonlinearities and complexities involved in describing carbon metabolism, the trace element approach relies on accurate estimates of the elimination of a nonessential element that can be described by a first-order linear equation (Rowan and Rasmussen 1995). We present age-specific concentrations of ^{137}Cs in 19 species of fish and their food, use these data and growth rate to estimate consumption, then insert these consumption estimates into the general bioenergetics equation of Hewett and Johnson (1992) to solve for activity. All estimates reflect annual averages obtained from a single sampling. Finally, we test the sensitivity of the model to its parameters and discuss our results in relation to reproductive ecology and fish management.

This method is widely applicable because ^{137}Cs is detectable in all aquatic organisms through gamma spectrometry, and with ICP-mass spectrometry ^{137}Cs can now be measured precisely. Cesium is unique among inorganic elements in that it bioconcentrates in phytoplankton (10- to 1000-fold over environmental concentrations). Thereafter, cesium is transferred through ingestion (direct uptake is negligible for fish) and biomagnifies (about a 2-fold increase with each trophic level) (Rowan and Rasmussen 1994).

Methods

Combining the mass balances of growth and ^{137}Cs, consumption can be estimated as follows.
The mass balance of growth is

$$(2) \quad w_t = w_0 e^{Gt}$$

and the mass balance of ^{137}Cs is

$$(3) \quad dQ/dt = \alpha[^{137}Cs_f]Cw_t - (E + D)Q$$

Combining eqs. 2 and 3 gives

$$(4) \quad dQ/dt = \alpha[^{137}Cs_f]Cw_0e^{Gt} - (E + D)Q$$

and integrating gives

$$(5) \quad Q_t = \frac{\alpha[^{137}Cs_f]Cw_0(e^{Gt} - e^{-(E+D)t}) + Q_0e^{-(E+D)t}}{(G + E + D)}$$

If spawning occurs during the period modelled, the ^{137}Cs lost in eggs and sperm should be subtracted:

$$(6) \quad Q_t = \frac{\alpha[^{137}Cs_f]Cw_0(e^{Gt} - e^{-(E+D)t}) + Q_0e^{-(E+D)t} - Q_g}{(G + E + D)}$$

We can now solve for consumption:

$$(7) \quad C = \frac{(Q_t - Q_0e^{-(E+D)t} + Q_g)(G + E + D)}{\alpha[^{137}Cs_f]w_0(e^{Gt} - e^{-(E+D)t})}$$

where C is the consumption rate (g food·g body mass^{-1}·d^{-1}), Q_t is the ^{137}Cs burden (Bq) at time t (days), Q_0 is the initial ^{137}Cs burden (Bq), Q_g is the gonadal ^{137}Cs burden released at spawning, G is the specific growth rate (g·g^{-1}·d^{-1}), E is the elimination rate of ^{137}Cs (Bq·Bq^{-1}·d^{-1}), D is the radioactive decay of ^{137}Cs (Bq·Bq^{-1}·d^{-1}), [^{137}Cs$_f$] is the concentration of Cs in food (Bq·kg^{-1}), α is the assimilation efficiency of ^{137}Cs from food (fraction), and w_0 is the initial body mass (kg). With this radiotracer approach, validated by comparisons with consumption estimates from gut contents and clearance rates (Forseth et al. 1992), consumption is estimated independent of metabolic costs and is not limited by fish size. This model can be solved from a single sampling (annual average) or with two samplings over shorter time periods (weeks to months). By inserting these cesium-based consumption estimates into the standard bioenergetics equation (eq. 1) and using the resting metabolism, specific dynamic action, egestion, and excretion parameters commonly used (Hewett and Johnson 1992), we can solve for activity, avoiding any assumptions of swimming speed or arbitrary assignments of integer multipliers.

To solve eq. 7, one needs age-specific measures of the ^{137}Cs burden in fish at the beginning and end of the modelled period and, if spawning occurs, the ^{137}Cs burden lost in gonadal tissue. Concentrations of ^{137}Cs in fish diet, the assimilation efficiency of ^{137}Cs from that food, fish growth, and the elimination of ^{137}Cs during the modelled period are also required. All of our calculations were integrated over 365 days and thus reflect annual averages.

Fish were collected from the Ottawa River during 1993 using multi-mesh experimental gill nets and rod and reel. Fish were aged using scales and other hard parts. Whole fish were dried, ashed at 450°C, and composited by age-class, and average whole body ^{137}Cs concentration was measured by gamma spectrometry (Table 1). Initial ^{137}Cs concentrations and body mass were obtained from adjacent cohorts or through age versus ^{137}Cs or mass regressions (Table 1). Because our calculations were done on an annual basis, we included

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Table 1. ^{137}Cs concentrations, body mass, growth, and elimination (input data for eq. 7), as well as estimates of consumption and activity for fish from the Ottawa River and other systems.

	n	Age (years)	$[^{137}Cs]_t$ (Bq·kg^{-1})	$[^{137}Cs]_0$ (Bq·kg^{-1})	w_t (g)	w_0 (g)	G (g·g^{-1}·d^{-1})	E (Bq·Bq^{-1}·d^{-1})	C (g·g^{-1}·d^{-1})	A
Ottawa River										
Blacknose shiner (*Notropis heterolepis*)										
	20	1–2 m	3.62	3.62[a]	2.8	1.5	0.0017	0.0038	0.0398	1.777
Cisco (*Coregonus artedii*)										
	1	0 i	1.06		5.9					
	1	1 i	0.93	1.15[b]	13.3	6.2[b]	0.0021	0.0055	0.0186	0.988
	4	2 m	2.91	1.55[b]	26.2	12.2[b]	0.0021	0.0030	0.0454	3.341
	2	3 m	2.88	2.06[b]	47.0	27.9[b]	0.0014	0.0028	0.0368	3.186
	1	4 m	2.24	2.56[b]	96.0	52.6[b]	0.0017	0.0026	0.0260	2.487
	1	5 m	3.83	3.07[b]	124.9	86.2[b]	0.0010	0.0025	0.0278	3.078
Emerald shiner (*Notropis antherinoides*)										
	734	1 i	2.59		0.2					
	54	2 m	3.86	2.97[b]	1.5	0.3[b]	0.0041	0.0042	0.0911	3.446
	153	3 m	3.70	3.40[b]	3.2	1.4[b]	0.0023	0.0038	0.0645	2.975
	15	4 m	4.67	3.83[b]	4.8	3.0[b]	0.0013	0.0036	0.0662	3.556
	2	5 m	4.32	4.26[b]	8.1	5.2[b]	0.0012	0.0034	0.0569	3.307
Johnny darter (*Etheostoma nigrum*)										
	30	1 i	4.19		0.3		0.0138			
	3	2 m	4.86	4.19	0.6	0.3	0.0023	0.0046	0.0511	2.258
Lake whitefish (*Coregonus clupeaformis*)										
	1	4 m	7.70	6.82[b]	487.0	326.0[b]	0.0011	0.0022	0.0238	3.291
	1	6 m	9.47	8.58[b]	1124.6	752.7[b]	0.0011	0.0020	0.0274	4.634
Logperch (*Percina caprodes*)										
	1	1 i			0.4					
	79	2 m	4.69	4.65[c]	0.8	0.4	0.0020	0.0045	0.0460	2.136
	2	4 m	4.61	4.65[c]	3.0	1.7	0.0017	0.0038	0.0384	2.360
Northern pike (*Esox lucius*)										
	1	1 i	8.86		89.8					
	1	3 m	8.93	9.27[b]	544.3	234.6[b]	0.0023	0.0021	0.0259	2.401
	5	4 m	13.16	12.48[b]	872.6	546.6[b]	0.0013	0.0020	0.0259	2.864
	2	5 m	15.34	15.69[b]	1614.3	983.4[b]	0.0014	0.0019	0.0266	3.243
Rock bass (*Ambloplites rupestris*)										
Spring	3	3 i	6.24	4.51[b]	36.5	16.8[b]	0.0021	0.0034	0.0325	2.813
	3	4 mf	7.85	6.17[b]	95.3	41.0[b]	0.0023	0.0027	0.0377	4.736
	4	5 mf	9.53	7.82[b]	128.1	77.1[b]	0.0014	0.0025	0.0361	5.263
	6	6 mf	10.66	9.48[b]	182.2	126.0[b]	0.0010	0.0024	0.0278	4.281
	4	7 mf	13.12	11.14[b]	260.9	188.1[b]	0.0009	0.0023	0.0329	5.445
Summer	7	4 m	5.96	6.13[c]	97.8	46.4[b]	0.0020	0.0026	0.0315	3.908
	4	5 m	6.63	6.13[c]	135.5	94.4[b]	0.0010	0.0024	0.0233	3.414
	2	6 m	5.81	6.13[c]	193.8	142.4[b]	0.0008	0.0024	0.0197	3.091
Shorthead redhorse (*Moxostoma macrolepidotum*)										
	1	3 i	9.08		181.4					
	1	5 m	3.87	5.88[c]	609.2	361.5[b]	0.0014	0.0021	0.0277	3.541
	8	6 m	5.68	5.88[c]	988.8	661.2[b]	0.0011	0.0020	0.0352	5.308
	1	7 m	5.34	5.88[c]	1552.8	1027.5[b]	0.0011	0.0019	0.0264	4.256
Smallmouth bass (*Micropterus dolomieui*)										
Spring		2 i								
	3	3 i	6.78	6.78[a]	104.2	33.9[b]	0.0031	0.0028	0.0192	2.028
	5	4 i	6.62	5.40[b]	163.2	93.1[b]	0.0015	0.0024	0.0134	1.929
	6	5 m	11.03	8.66[b]	274.5	181.8[b]	0.0011	0.0023	0.0199	3.853
	3	6 m	17.58	11.92[b]	500.2	299.9[b]	0.0014	0.0022	0.0345	8.477
	3	7 m	17.60	15.18[b]	606.0	447.4[b]	0.0008	0.0021	0.0268	7.328
Fall	2	0 i	3.30		5.3					
	1	2 i	7.47	5.31[b]	109.4	43.5[b]	0.0025	0.0026	0.0195	2.002
	2	3 i	6.47	6.16[b]	135.1	91.5[b]	0.0011	0.0024	0.0109	1.288
	2	5 m	8.68	7.86[b]	410.0	255.2[b]	0.0013	0.0022	0.0153	2.838

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Table 1 (*continued*).

	n	Age (years)	$[^{137}Cs]_r$ (Bq·kg^{-1})	$[^{137}Cs]_0$ (Bq·kg^{-1})	w_r (g)	w_0 (g)	G (g·g^{-1}·d^{-1})	E (Bq·Bq^{-1}·d^{-1})	C (g·g^{-1}·d^{-1})	A
	2	6 m	10.90	8.71[b]	561.8	370.9[b]	0.0011	0.0021	0.0188	4.456
	1	7 m	8.95	9.57[b]	673.6	509.2[b]	0.0008	0.0020	0.0118	3.038
	2	8 m	13.65	10.42[b]	774.6	670.0[b]	0.0004	0.0020	0.0188	5.582
	3	9 m	11.49	11.27[b]	973.3	853.3[b]	0.0004	0.0019	0.0132	4.205
	1	10 m	11.79	12.12[b]	1379.0	1059.3[b]	0.0007	0.0019	0.0148	5.240
Spottail shiner (*Notropis hudsonius*)										
	54	1 i	2.08		0.5					
	5	2 m			2.1					
	33	3 m	3.05	2.57[b]	3.8	2.1	0.0017	0.0037	0.0476	2.302
Walleye (*Stizostedion vitreum*)										
	3	0 i	6.00		37.2					
	1	1 i	10.19	6.98[b]	109.6	17.0[b]	0.0031	0.0026	0.0248	2.480
	4	2 i	8.90	8.59[b]	189.1	106.3[b]	0.0016	0.0024	0.0133	1.380
	4	3 i	12.58	10.20[b]	274.7	195.5[b]	0.0009	0.0023	0.0161	2.018
	5	4 i	13.68	11.81[b]	362.1	284.7[b]	0.0007	0.0022	0.0154	2.139
	4	5 i	13.47	13.42[b]	464.1	374.0[b]	0.0006	0.0021	0.0135	1.909
	4	6 m	18.44	15.04[b]	539.0	463.2[b]	0.0004	0.0021	0.0206	3.151
	2	9 m	20.89	16.65[b]	826.9	552.5[b]	0.0004	0.0020	0.0197	3.620
White sucker (*Catostomus commersoni*)										
	22	1 i	1.73		4.3					
	1	2 i	2.85		80.8	2.9[b]	0.0091	0.0032	0.0346	1.369
	2	5 m	4.64	4.05[b]	1289.9	886.9[b]	0.0010	0.0019	0.0239	3.670
Yellow perch (*Pera flavescens*)										
Spring	224	1 i	5.10		1.7					
	78	2 i	5.62	5.01[b]	9.2	1.9[b]	0.0043	0.0036	0.0233	1.007
	8	3 i	6.51	5.71[b]	32.2	8.2[b]	0.0037	0.0031	0.0232	1.439
	5	4 m	9.45	6.42[b]	74.7	28.0[b]	0.0027	0.0027	0.0229	1.904
	5	5 m	13.42	9.79[b]	127.7	78.3[b]	0.0013	0.0025	0.0242	2.623
	1	6 m	17.76	13.25[b]	179.8	128.7[b]	0.0009	0.0024	0.0282	3.490
	4	7 m	18.08	16.70[b]	223.6	179.0[b]	0.0006	0.0023	0.0239	3.178
	2	8 m	24.63	20.15[b]	284.9	229.4[b]	0.0006	0.0022	0.0335	4.752
Fall	9	2 i	7.52	5.27[b]	14.3	3.9[b]	0.0035	0.0035	0.0283	1.590
	27	3 i	8.11	6.77[b]	41.0	17.1[b]	0.0024	0.0028	0.0228	1.874
	3	4 m	9.67	8.28[b]	95.7	53.1[b]	0.0016	0.0026	0.0187	1.791
	1	8 m	14.26	14.29[b]	302.8	254.5[b]	0.0005	0.0022	0.0175	2.451
	1	9 m	18.61	15.80[b]	392.9	304.9[b]	0.0007	0.0022	0.0258	3.858
White Oak Lake										
Bluegill (*Lepomis macrochirus*)										
		3	1550.00		90.6					
	33	4 m	2061.00	1550.00	109.2	90.6	0.0005	0.0050	0.0221	3.260
Carp (*Cyprinus carpio*)										
	1	0 i	654.90		53.0					
	3	1 i	792.91	654.90	167.3	47.7[b]	0.0034	0.0044	0.0289	1.097
	9	2 i	930.92	843.50[c]	265.6	166.0[b]	0.0013	0.0040	0.0256	1.369
	2	3 i	806.60	843.50[c]	414.5	284.2[b]	0.0010	0.0039	0.0215	1.251
Lake Erie										
Coho salmon (*Oncorhynchus kisutch*)										
	4	3 m	1.26	1.26[a]	2778.0	800.2[b]	0.0034	0.0021	0.0265	5.528
Lake Michigan										
Coho salmon (*Oncorhyncus kisutch*)										
	5	3 m	15.58	15.58[a]	3527.8	1031.1[b]	0.0034	0.0013	0.0151	3.825
Chinook salmon (*Oncorhynchus tshawytscha*)										
	14	3 m	10.51	9.56[b]	6337.1	1935.0[b]	0.0033	0.0012	0.0129	4.105
	1	3 m	16.43	15.00	4960.0	1935.0[b]	0.0026	0.0012	0.0131	4.528
Lake trout (*Salvelinus namaycush*)										
	1	4 i	12.17	12.17[a]	2100.0	1395.0[b]	0.0011	0.0012	0.0056	2.270

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Table 1 (*concluded*).

	n	Age (years)	$[^{137}Cs]_t$ (Bq·kg^{-1})	$[^{137}Cs]_0$ (Bq·kg^{-1})	w_t (g)	w_0 (g)	*G* (g·g^{-1}·d^{-1})	*E* (Bq·Bq^{-1}·d^{-1})	*C* (g·g^{-1}·d^{-1})	*A*
Great Slave Lake										
Christie Bay										
Lake trout (*Salvelinus namaycush*)										
	1	3 i	3.89	4.71[c]	250.0	100.5[b]	0.0025	0.0014	0.0141	1.955
	1	4 i	5.22	4.71[c]	500.0	285.6[b]	0.0015	0.0013	0.0074	1.308
	3	5 i	4.63	4.71[c]	716.7	488.2[b]	0.0011	0.0013	0.0046	0.915
	3	6 i	5.44	4.71[c]	1033.3	779.3[b]	0.0008	0.0012	0.0063	2.025
	3	7 i	4.19	4.71[c]	1150.0	925.3[b]	0.0006	0.0012	0.0028	0.778
	2	8 i	4.92	4.71[c]	1400.0	1177.1[b]	0.0005	0.0012	0.0040	1.585
	2	9 i	4.48	4.71[c]	1600.0	1388.0[b]	0.0004	0.0011	0.0029	1.245
	3	10 mm	5.53	5.33[b]	2283.3	1628.0[b]	0.0009	0.0011	0.0062	3.188
	2	10 mf	5.98	6.93[c]	2400.0	1818.4[b]	0.0008	0.0011	0.0075	4.187
	1	11 mm	5.70	6.22[b]	2900.0	2242.0[b]	0.0007	0.0011	0.0049	2.709
	4	11 mf	6.93	6.93[c]	3090.0	2403.3[b]	0.0007	0.0011	0.0070	3.758
	2	12 mm	7.40	7.11[b]	3575.0	2925.6[b]	0.0005	0.0010	0.0066	4.718
	4	12 mf	6.62	6.93[c]	3875.0	3080.1[b]	0.0006	0.0010	0.0061	3.488
	3	13 mf	7.97	6.93[c]	4966.7	4020.9[b]	0.0006	0.0010	0.0089	6.973
	3	14 mf	7.31	6.93[c]	5883.0	4837.9[b]	0.0005	0.0010	0.0070	5.727
	2	15 mf	6.36	6.93[c]	6900.0	5751.6[b]	0.0005	0.0010	0.0048	3.706
Northern pike (*Esox lucius*)										
	1	9 mf	3.77	4.42[c]	1250.0	801.3[b]	0.0008	0.0014	0.0108	1.358
	3	10 mf	4.55	4.42[c]	1400.0	1061.3[b]	0.0007	0.0014	0.0112	1.476
	2	10 mm	4.31	4.42[c]	1500.0	1061.3[b]	0.0007	0.0014	0.0075	1.009
	1	12 mm	4.92	4.42[c]	2200.0	1713.0[b]	0.0006	0.0013	0.0080	1.199
	1	15 mf	4.37	4.42[c]	4200.0	3045.5[b]	0.0005	0.0012	0.0113	1.899
McLeod Bay										
Northern pike (*Esox lucius*)										
	1	11 mf	22.16	22.89[c]	1800.0	1364.6[b]	0.0006	0.0014	0.0083	1.092
	1	12 mf	23.61	22.89[c]	2100.0	1713.0[b]	0.0006	0.0013	0.0082	1.120
Western basin										
Northern pike (*Esox lucius*)										
	1	10 m	1.78	1.78[a]	1550.0	1061.3[b]	0.0007	0.0014	0.0172	2.504

Note: i, immature; m, mature; mf, mature female; mm, mature male.

[a] $[^{137}Cs]_t = [^{137}Cs]_0$.

[b] Calculated from regression.

[c] Average.

in our model the loss of ^{137}Cs from mature fish from the release of gonadal tissue during spawning. Yellow perch, rock bass, smallmouth bass, and walleye eggs were removed from ripe female fish, gonadal/somatic index (GSI) was calculated, and ^{137}Cs concentrations were determined by gamma spectrometry (Table 2). We also obtained published concentrations of ^{137}Cs in whole fish and eggs for coho salmon, chinook salmon, brown trout (*Salmo trutta*), carp, northern pike, and shorthead redhorse (Nelson et al. 1972; Bowen 1974), along with additional data on GSI (Bowen 1974; Scott and Crossman 1973) (Table 2). For males, gonadal ^{137}Cs concentrations were not available and were assumed to be equal to whole body concentrations. Because male GSI is usually low (always less than 5%), this assumption should introduce little uncertainty to the results. For all cyprinids, we used the carp egg to whole body ^{137}Cs value; for logperch and johnny darter, the mean percid value was used; for white sucker, the shorthead redhorse value was used; and we used the mean salmonid value for coregonids. The average egg/whole body ^{137}Cs concentration ratio was 0.56 ± 0.07 (Table 2). Composite samples were assumed to consist of 50% males and 50% females, and gonadal burdens were calculated accordingly.

Fish diet was determined by examining gut contents (Table 3). Forage fish consumed by piscivores and omnivores were collected and processed as described above. Invertebrates identified in gut contents were collected from the Ottawa River, sorted into taxa, and dried, and the ^{137}Cs concentration was determined by gamma spectrometry (Table 4). Zooplankton were also collected biweekly using a 151-μm net and dried, and the ^{137}Cs concentration was determined by gamma spectrometry (Table 4). The concentration of ^{137}Cs in the fish diet was determined by weighting food ^{137}Cs concentrations according to the proportions of food items in gut contents (Table 3). Assimilation efficiencies (α) were calculated in a similar manner (Table 3). The assimilation efficiency (α, fraction) of ^{137}Cs from food is 0.638 ± 0.058 from invertebrate tissue (Kevern 1966; Kolehmainen 1972; Hewett and Jefferies 1978; Forseth et al. 1992) and 0.690 ± 0.013 from fish tissue (Kolehmainen 1972; Aoyama and Inoue 1973; Aoyama et al. 1978; Forseth et al. 1992).

Growth was determined by comparing adjacent cohorts ($\ln(W_i/W_{i-1})/365$) or through age versus mass regressions. The elimination rate (*E*) of radiocesium from fish has been shown to be species independent and can be described (Rowan and Rasmussen 1995) by $\ln E = -6.583 - 0.111(\ln W) + 0.093T$, $r^2 = 0.966$, $SE_{est} = 0.130$, $n = 20$, $P < 0.0005$, where *W* is mass (g) and *T* is temperature (°C). Ottawa River annual water temperature data were obtained biweekly during the ice-free season of 1993.

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Published data on ^{137}Cs concentrations in coho salmon, chinook salmon, and lake trout and their food were obtained for Lake Michigan (Nelson et al. 1972; Waller et al. 1974; Spigarelli 1976), for coho salmon and their food for Lake Erie (Marshall et al. 1975; Joshi 1984), for lake trout and northern pike and their food for Great Slave Lake (Elliott et al. 1981), and for bluegill and carp and their food from White Oak Lake (Kevern 1966; Kolehmainen 1972, 1974) (Tables 1, 3). We also obtained growth rate (Miller and Kennedy 1948; Kennedy 1954; Kevern 1966; Kolehmainen 1972, 1974; Reinert and Bergman 1974; Stewart et al. 1981, 1983; Clark et al. 1984), diet (Miller and Kennedy 1948; Kennedy 1954; Kevern 1966; Kolehmainen 1972, 1974; Stewart et al. 1981, 1983), and annual water temperature data for these systems (Rawson 1950; Ayers 1962; Burns 1970; Kolehmainen 1974). These data were treated as above except that separate calculations were made for male and female fish from Great Slave Lake (Tables 1, 3).

We then calculated consumption rates from the above data using eq. 7 and input these values into eq. 1 to solve for activity. As bioenergetics parameters for resting metabolism, specific dynamic action, egestion, and excretion were unavailable for many fish in our data, we borrowed values from similar fish (Hewett and Johnson 1992): lake whitefish (from cisco), emerald shiner (dace), blacknose shiner (dace), spottail shiner (dace), johnny darter (yellow perch), logperch (yellow perch), rock bass (bluegill), white sucker (dace), and shorthead redhorse (dace).

Table 2. Female and male gonadal somatic indices (GSI) and the ratio of egg ^{137}Cs to whole body ^{137}Cs (fraction).

	GSI		^{137}Cs ratio
	Female	Male	(egg/whole body)
Blacknose shiner	0.15	0.03	0.438
Bluegill	0.05	0.01	0.458
Cisco	0.15	0.03	0.421
Emerald shiner	0.15	0.03	0.438
Johnny darter	0.16	0.03	0.423
Logperch	0.16	0.03	0.423
Lake trout	0.25	0.05	0.421
Lake whitefish	0.10	0.02	0.421
Northern pike	0.18	0.02	1.728
Rock bass	0.05	0.01	0.510
Shorthead redhorse	0.10	0.02	1.270
Smallmouth bass	0.05	0.01	0.355
Spottail shiner	0.15	0.03	0.438
Walleye	0.15	0.03	0.511
White sucker	0.10	0.02	1.270
Yellow perch	0.16	0.03	0.423

Note: ^{137}Cs concentration in male gonadal tissue is assumed to be equal to whole body levels of ^{137}Cs.

Results

Using the ^{137}Cs mass balance approach, we estimated annual average, age-specific consumption rates and energetic costs of activity for 14 species of fish from the Ottawa River and augmented these data with information for an additional 5 species from published data on ^{137}Cs concentrations (Table 1). We found that activity for immature fish ($n = 27$ age-classes, 8 species) was usually between 1- and 2-fold resting metabolism (1.63 ± 0.10) and was not significantly different from published values (1.40 ± 0.08) (Hewett and Johnson 1992) (Figs. 1, 2). However, mature fish ($n = 70$ age-classes, 18 species) had higher activities than immature fish (3.53 ± 0.17, $P < 0.0005$) and these activities were higher ($P < 0.0005$) than published estimates (1.51 ± 0.06) (Hewett and Johnson 1992) (Figs. 1, 2). Our activity estimates for mature fish were always 1.4- to 4.8-fold higher than the integer multipliers (Winberg 1956; Kitchell et al. 1977; Hewett and Johnson 1992) (Fig. 1) and up to 4.1-fold higher than those estimates based on swimming speed (Hewett and Johnson 1992) (Fig. 2). We also found higher activity (but not significantly higher) in female northern pike (1.58 ± 0.16) than in male northern pike (1.10 ± 0.09) (Fig. 2) and in female lake trout (4.64 ± 0.57) than in male lake trout (3.54 ± 0.61) from Christie Bay, Great Slave Lake (Fig. 2). These results suggest that activity in mature fish is elevated by reproductive efforts.

Between-system intraspecific variability could be compared for only a few species and systems: coho salmon from lakes Erie and Michigan, lake trout from Lake Michigan and Christie Bay, Great Slave Lake, and northern pike from the Ottawa River and three basins of Great Slave Lake. Our results suggest that between-system variability within species can exceed 2-fold. Coho salmon activity differed by 1.4-fold between the two very different systems (5.53 for Lake Erie, 3.83 for Lake Michigan) (Table 1, Fig. 2). Growth rates were similar but the greater consumption in Lake Erie was offset by higher resting metabolism at higher water temperatures. The activity of lake trout before first spawning in Lake Michigan (2.27) fell between the activity of immature and mature lake trout from Great Slave Lake (1.40 ± 0.18 and 4.27 ± 0.45), with similar growth and consumption in the two systems (Table 1, Fig. 2). The activities of northern pike from McLeod (1.11 ± 0.01) and Christie (1.39 ± 0.15) bays of Great Slave Lake were not significantly different from each other but were lower than the activity of pike from the Ottawa River (2.84 ± 0.24) and the western basin (2.50) of Great Slave Lake ($P < 0.0005$) (Fig. 2). Higher consumption rates in the latter two systems were largely responsible for the higher activity-related costs. Others have also found large differences in activity related to parameters such as forage abundance and water clarity (Boisclair and Rasmussen 1996). Clearly, more within-species comparisons are required to assess the generality of the activity parameters presented here.

Our results suggest that the metabolic costs related to activity increase for reasons other than routine costs associated with swimming. Activities of 1.13 for immature northern pike estimated from laboratory swimming-speed relationships (Belvelhimer et al. 1985) and 1.15 from heart rate telemetry (Lucas et al. 1991) are in excellent agreement with estimates for male northern pike from Christie Bay of Great Slave Lake (1.10 ± 0.09) but are 1.4-fold lower than estimates for female northern pike from the same bay (1.58 ± 0.16) (Table 1, Fig. 2). Female northern pike can have consumption rates up to 1.3-fold greater than male northern pike to support gonadal growth (Diana 1980), in excellent agreement with the 1.4-fold greater consumption rates in female (0.011 ± 0.000) than in male northern pike (0.008 ± 0.000) ($P < 0.001$) from Christie Bay (Table 1, Fig. 2). Lake trout show similar patterns. Activity related to swimming averages 1.62 ± 0.01 (Hewett and Johnson 1992) for immature lake trout (ages 3+ to 9+), in good agreement with our estimate of 1.40 ± 0.18, but for mature fish (ages 10+ to 15+), swimming speed estimates of activity are only 1.69 ± 0.01 (Hewett and Johnson 1992) compared with our estimate of 4.27 ± 0.45 (Fig. 2).

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Table 3. Fish diet, ^{137}Cs concentrations (Bq·kg wet weight^{-1}) in diet, and assimilation efficiency (α, fraction) of ^{137}Cs from diet.

	^{137}Cs	α	Diet
			Ottawa River
Blacknose shiner	0.845	0.638	50% insects and crustaceans, 50% zooplankton
Cisco			
Age 1+ to 4+	0.589	0.638	100% zooplankton
Age 5+	0.867	0.638	50% zooplankton, 50% hexagenia
Emerald shiner	0.589	0.638	100% zooplankton
Johnny darter	1.101	0.638	100% insects and crustaceans
Lake whitefish	1.836	0.651	75% insects and crustaceans, 25% YOY yellow perch
Logperch	1.101	0.638	100% insects and crustaceans
Northern pike	3.743	0.685	60% YOY yellow perch, 20% cisco, 10% johnny darter, 10% crayfish
Rock bass			
Age 3+ to 4+	1.745	0.639	40% crayfish, 30% anisoptera, 20% gastropods, 7% tricoptera, 3% YOY yellow perch
Age 5+ to 7+	2.217	0.638	66% crayfish, 27% anisoptera, 6% miscellaneous insects and crustaceans, 1% YOY yellow perch
Shorthead redhorse	1.101	0.638	100% insects and crustaceans
Smallmouth bass	3.155	0.660	50% crayfish, 42% YOY yellow perch, 8% miscellaneous insects and crustaceans
Spottail shiner	0.589	0.638	100% zooplankton
Walleye	3.946	0.690	97% YOY yellow perch, 3% hexagenia
White sucker	1.101	0.638	100% insects and crustaceans
Yellow perch			
Age 1+ to 3+	2.927	0.664	50% YOY yellow perch, 26% crayfish, 21% gastropod, 3% miscellaneous insects and crustaceans
Age 4+ to 9+	3.627	0.681	77% YOY yellow perch, 10% miscellanous insects and crustaceans, 4% crayfish, 4% johnny darter, 3% gastropods, 2% logperch
			White Oak Lake
Bluegill	2948.4	0.183	85% insects and crustaceans, 10% detritus, 5% plants
Carp	540.9	0.362	60% detritus, 40% algae
			Lake Erie
Coho salmon	0.384	0.690	100% smelt
			Lake Michigan
Coho salmon	7.045	0.690	93% adult alewife, 5% other forage fish, 2% YOY alewife
Chinook salmon			
1973	5.380	0.690	93% adult alewife, 5% forage fish, 2% YOY alewife
1971	7.045	0.690	93% adult alewife, 5% forage fish, 2% YOY alewife
Lake trout	7.423	0.690	63% adult alewife, 30% forage fish, 7% YOY alewife
			Great Slave Lake
Christie Bay			
Lake trout	3.071	0.690	100% cisco
Northern pike	2.053	0.690	100% forage fish
McLeod Bay			
Northern pike	12.987	0.690	100% forage fish
Western basin			
Northern pike	0.454	0.690	100% forage fish

Note: YOY, young of the year.

Yellow perch, rock bass, and smallmouth bass were collected from the Ottawa River on two occasions, allowing comparison of annual activity estimates from the independent data sets for these species. We found no significant differences in activity calculated from spring and fall data for immature (1.22 ± 0.22 vs. 1.73 ± 0.14) or mature (3.19 ± 0.47 vs. 2.70 ± 0.61) yellow perch or for immature (1.98 ± 0.05 vs. 1.65 ± 0.36) or mature (6.55 ± 1.39 vs. 4.23 ± 0.46) smallmouth bass. There were significant differences between mature rock bass collected during spring and summer (4.93 ± 0.26 vs. 3.47 ± 0.24). However, in four of the five tests, we did find agreement between activity estimates, suggesting that one sampling may be sufficient to adequately describe the annual average activity.

We tested the sensitivity of our activity estimates to uncertainty in the parameters in eq. 7 for a large, slow-growing fish (age 6+ walleye) and a small, fast-growing fish (age 3+ emerald shiners) (Fig. 3). Each parameter in eq. 7 was increased and decreased by 10%, and the resultant change in activity was calculated. Activity estimates are most sensitive to the concentration of ^{137}Cs at the end of the time period modelled, with activity changes in excess of 10% for a 10% change in ^{137}Cs concentration. Activity is also sensitive to uncertainty in the uptake parameters (about a 10% change for a 10% change in assimilation efficiency, ^{137}Cs concentration in food, or consumption rate) and moderately sensitive to elimination and body size (4–7% change for a 10% change in E and w). Activity

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estimates are sensitive to growth for fast-growing fish and initial ^{137}Cs concentrations for slow-growing fish (about a 7% change for a 10% change in these parameters). Activity estimates are not sensitive to losses of ^{137}Cs through gonadal tissue (less than 1% change for a 10% change in gonadal concentration of ^{137}Cs and GSI).

Our method of estimating activity by difference results in the accumulation of all measurement errors into that term. We also recognize that static parameters were used for specific dynamic action, egestion, and excretion and that oversimplification of these processes could lead to errors in our activity estimates. Certainly, improvements in modelling resting metabolism, specific dynamic action, egestion, and excretion would lead to improvements in activity estimates by the ^{137}Cs method proposed.

It could be argued that some systemic bias in any of the important parameters listed above is responsible for the patterns in our activity estimates: mature > immature. Because ^{137}Cs concentration can be measured precisely in fish and their food, the most likely source of uncertainty in our approach stems from diet composition and assimilation efficiency. However, the assimilation efficiency of ^{137}Cs from food that contains little clay is not variable, ranging from 60 to 70%, and it does not represent the 2- to 4-fold variation necessary to explain our results. Underestimation of the concentration of ^{137}Cs in the diet of older fish by 2- to 4-fold (1 trophic level) could lead to the pattern, but for most species we saw no change in diet composition or prey size with age, let alone a shift in trophic level (Table 3). For several species (rock bass and yellow perch), we saw distinct diet shifts and higher ^{137}Cs concentrations in older fish, but we still observed significantly higher activity in mature fish. The ^{137}Cs method, however, does require good estimates of diet composition, concentrations of ^{137}Cs in the diet items, and assimilation efficiency of ^{137}Cs from the food. We are currently exploring the use of stable nitrogen isotopes to quantify trophic position and diet composition (Cabana and Rasmussen 1994), as well as methodologies for estimating assimilation efficiency from gut contents.

Table 4. ^{137}Cs concentrations (Bq·kg wet weight^{-1}) in Ottawa River zooplankton, molluscs, insects, crustaceans, and young-of-the-year (YOY) yellow perch.

	^{137}Cs
Zooplankton (>151 μm)	0.589±0.077
Molluscs	
Campeloma	0.196
Eliptio complanata	0.139
Lymnaea	1.194
Physa	0.691
Planorbidae	0.977
Mean	0.639±0.187
Insects and crustaceans	
Amphipoda	1.518
Anisoptera	1.098
Baetidae	1.425
Orconectes propinquus	2.740±0.325
Hexagenia limbata	1.145
Leptophlebidae	0.904
Tricoptera	0.514
Mean (excluding crayfish)	1.101±0.136
YOY yellow perch	4.039±0.574

Note: Where applicable, values are given as the mean ± 1 SE.

Discussion

Activity and reproduction

Activities from conventional bioenergetics models (Hewett and Johnson 1992) are in excellent agreement with our ^{137}Cs-based estimates for immature fish, but our estimates for mature fish are consistently higher by 2- to 4-fold than either integer multipliers or swimming speed estimates. Activities of mature, nonspawning fish estimated through heart rate and electromyogram telemetry range from 1 to 2 (Priede and Young 1977; Weatherley et al. 1982), in agreement with swimming speed estimates or integer multipliers. These results indicate that the high activity costs observed in our study are associated with reproduction. This probably results from intensified activity during the spawning period; however, the seasonal breakdown of this increase would require the cesium mass balance to be incremented over the spawning period rather than on an annual basis. Energy costs associated with reproductive behaviour are presently very poorly known because of the difficulty in estimating activity costs in field studies. Potentially energy-demanding behaviours associated with reproduction are movements to and from breeding sites, defense of spawning sites, courtship and mating, and parental care (Keenleyside 1979; Wootton 1990). While some energetic studies have quantified the energy of long-distance spawning migrations (40–80% of the total energy budget) of anadromous species such as sockeye salmon (*Oncorhynchus nerka*) (Brett 1986) and American shad (*Alosa sapidissima*) (Leggett and Carscadden 1978; Glebe and Leggett 1981), little quantitative information is available for most of the species we are studying. A detailed energy budget for northern pike showed that both spawning males and females in Lac St. Anne, Alberta, suffered significant depletion of their somatic energy stores during the spring spawning period (Diana and MacKay 1979; Diana 1983) and that females depleted a greater proportion than males, in agreement with our activity estimates. Territorial defense by pupfish (Feldmeth 1983) entails about twice the cost of normal activity, and similar estimates have been made for threespine stickleback (*Gasterosteus aculeatus*) (Stanley and Wootton 1986). The energy costs of courtship and mating or parental care have rarely been estimated for any fish (Wootton 1990). The radiotracer approach to bioenergetic analysis has the potential to greatly facilitate the study of reproductive costs for wild populations because this radionuclide is so widely dispersed and easily analyzed.

It is also possible that during reproduction, fish metabolic rates increase for reasons other than swimming and that what we calculate as increased activity is actually increased basal metabolism. The ^{137}Cs bioenergetics method of estimating consumption does not rely on swimming speeds or metabolic costs, enabling the estimation of total metabolic costs as total M = assimilated $C - G$. Total metabolic costs have been estimated in this manner by Forseth et al. (1994). Even if basal metabolic rates are seasonally variable, our isotope approach can be used to test for differences in metabolic costs between immature and mature fish. If basal metabolism increases during

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Fig. 1. In situ annual average activity estimates for Ottawa River and White Oak Lake fish in comparison with integer multipliers (Hewett and Johnson 1992) for percids, cyprinids, centrarchids, and catostomids. ■, activity estimates from this study (vertical lines are 1 SE); □, published integer activity multipliers. All species were collected in spring (S), with additional collections of rock bass in summer (Su), and yellow perch and smallmouth bass in fall (F).

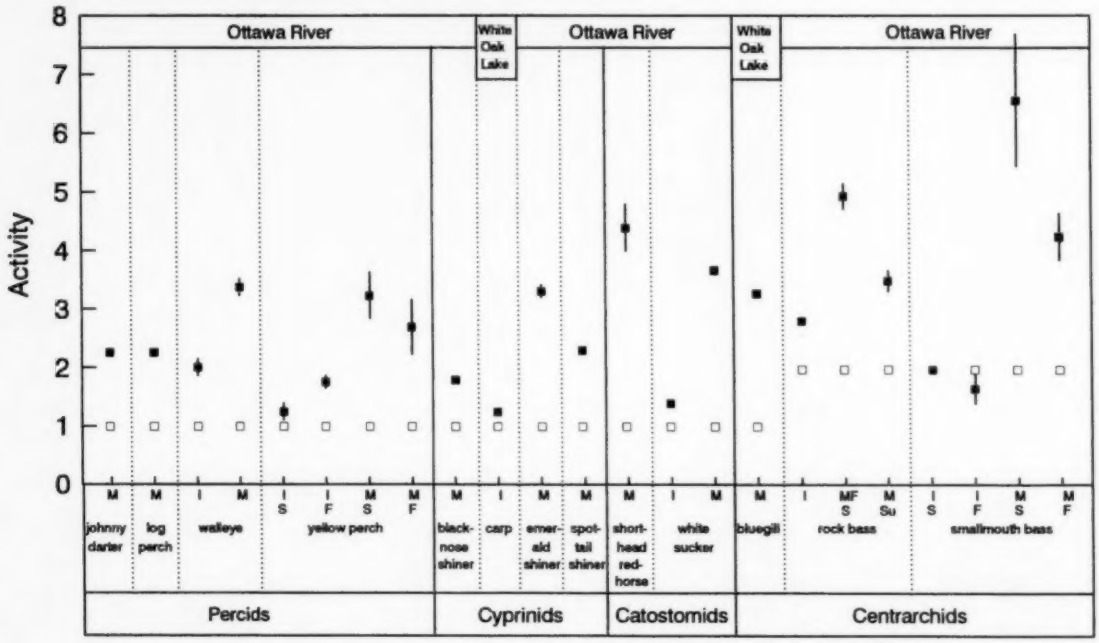

Fig. 2. In situ annual average activity estimates in comparison with estimates from swimming speed (Hewett and Johnson 1992) for coregonids, esocids, and salmonids. ■, activity estimates from this study (vertical lines are 1 SE); □, activities estimated from swimming speed.

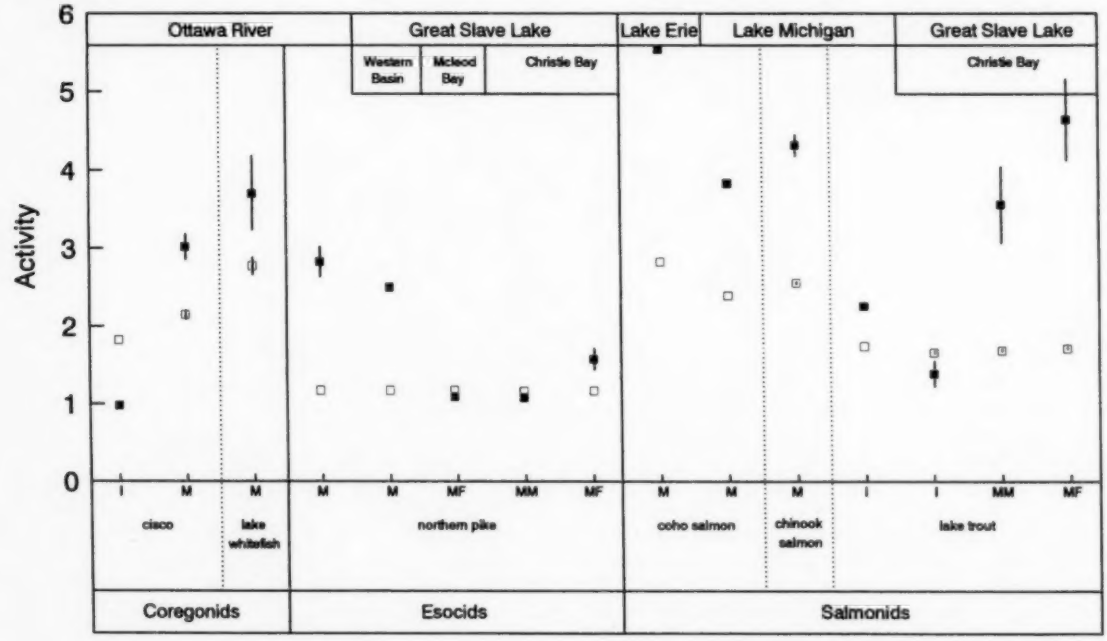

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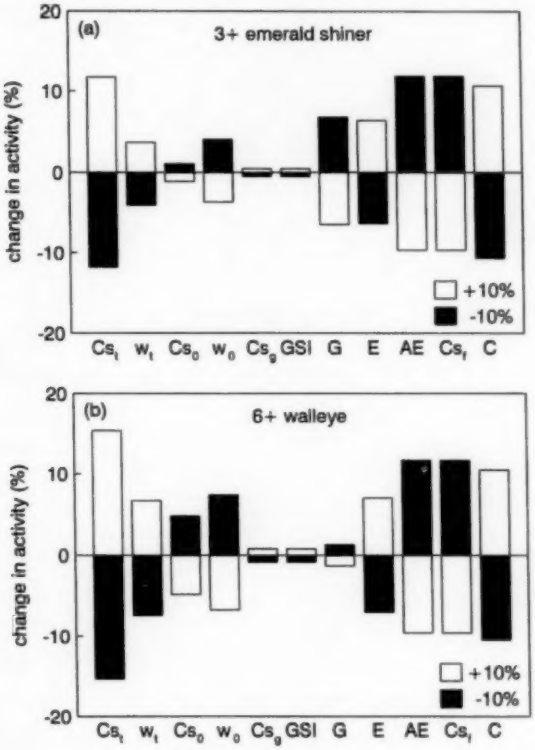
Fig. 3. Sensitivity of activity estimates to a 10% change in the parameters of eq. 7. (Cs_t and Cs_0, final and initial [Cs] in fish; w_t and w_0, final and initial body weight; Cs_g, [Cs] in gonadal tissue; GSI, gonadal/somatic index; G, growth; E, elimination; AE, assimilation efficiency; Cs_f, [Cs] in food; C, consumption rate).

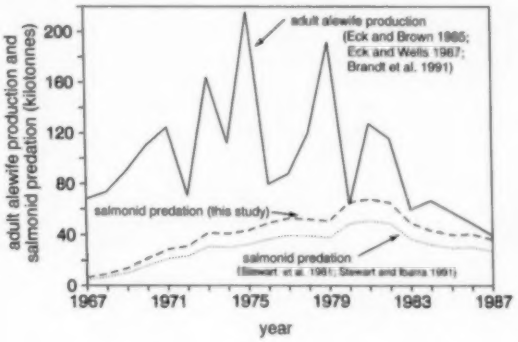


reproduction, our data still lead to the same conclusion: metabolic costs of mature fish are greater than those expected from allometric relationships, whereas those of immature fish can be explained by allometry.

Management implications

The underestimation of activity costs can have profound effects on systems managed with bioenergetics models. In the early 1980s, bioenergetics models were used to evaluate the predatory pressure exerted by salmonids on their main prey, the alewife (Stewart et al. 1981). Results of this work suggested that the stocking rates of salmonids in Lake Michigan were exerting high pressure on the forage base and Stewart et al. (1981) warned of a potential collapse of the alewife. If, as our results show, activity was underestimated by 1.5-fold for lake trout, 1.7-fold for coho salmon, and 1.7-fold for chinook salmon from Lake Michigan, it follows that consumption estimates would have been underestimated by 1.3-, 1.4-, and 1.3-fold, respectively. By weighting these estimates for consumption by species (Stewart et al. 1981; Stewart and Ibarra 1991), we can increase alewife consumption by salmonids by 33%, and our consumption estimates begin to approximate the production of adult alewife (Eck and Brown 1985; Eck and Wells 1987; Brandt et al. 1991) during the years following its collapse in 1980 (Fig. 4). Thus, the situation was even more severe than recognized (Stewart et al. 1981) and the failure of the alewife to recover is not surprising. If fisheries scientists are to convince governments that fisheries practices (stocking and catch quotas) threaten fisheries stability, consumption must not be underestimated. The use of ^{137}Cs bioenergetics provides an improvement in that respect.

Fig. 4. Adult alewife production and salmonid predation in Lake Michigan from 1967 to 1987. The failure of alewife to recover following its crash in 1980 is likely due to the predatory pressure of the salmonids that until now has been underestimated as a result of unrealistically low activity estimates.



In summary, ^{137}Cs is a widely dispersed and easily measured tracer that provides a simple and robust means of estimating in situ activity costs for fish. These activities can be used to strengthen existing management approaches based on bioenergetics that rely on laboratory-derived or assumed activity parameters. In addition, it is likely that the activity relationships identified here and linked to reproductive activities make in situ activity measurements the only means to avoid up to 2-fold errors in consumption or growth estimates for mature fish.

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When do fish with indirect development become juveniles?

Gordon H. Copp and Vladimír Kováč

Abstract: The transition between larval and juvenile development remains ill defined for fishes that undergo indirect development, with 0+ juveniles sometimes referred to as small adults. We examine this transition to determine whether 0+ juvenile roach, *Rutilus rutilus*, can be considered as small adults; we reanalysed data on the functional morphology, visual acuity, and microhabitat use of 0+ roach (15–55 mm standard length (SL)) in the rivers Great Ouse (United Kingdom) and Danube (Hungary and Slovakia). Factors related to swimming ability in 0+ roach attained adult levels at 35–45 mm SL, this corresponding with a temporary stabilisation in the development of visual acuity and with a significant (Mann–Whitney *U* test) microhabitat shift from protective cover in the littoral to the open channel. Contrary to current practice, which defines the start of the juvenile period in roach at 15–18 mm SL, we propose the 15–40 mm interval to be the final larval step, and that the juvenile period does not begin until morphological changes and structures characteristic of larval development (rapid allometric growth, remnants of finfold) have disappeared, all juvenile structures (complete scale cover, nasal septa, etc.) are present, and relative growth has stabilised.

Résumé : La transition entre les périodes de développement larvaire et juvénile reste mal défini pour les espèces de poissons d'eau douce à développement indirecte. Pour ces especes, les juvéniles de l'année (0+) sont en effet parfois considéres comme des petits adultes. Nous avons examiné cette transition pour déterminer si les juvéniles de l'année du gardon, *Rutilus rutilus*, sont des petits adultes, en reanalysant des données sur la morphologie fonctionnelle, l'acuité visuelle et les microhabitats de jeunes gardons (longueur standard (LS): 15–55 mm) dans la rivière Great Ouse (Grande Bretagne) et le Danube (Hongrie–Slovaquie). Chez le gardon 0+, les paramètres associés à la capacité de nage atteignent les valeurs caractéristiques de l'adulte pour une taille de 35 à 45 mm LS. Ceci s'accompagne d'une stabilisation temporaire dans le développement de l'acuité visuelle et d'un changement significatif (test *U* de Mann–Whitney) de microhabitat : les poissons quittent les zones protègées ou à courant lent de la zone littorale pour gagner le milieu du chenal. En contraste avec la pratique courante qui définit le début de la période juvénile pour une taille de 15 à 18 mm LS chez le gardon, nous proposons de considerer l'intervalle de 15 à 40 mm comme la dernière étape larvaire. Les critères marquant le début de la période juvénile sont alors la disparition des changements morphologiques et des structures caractéristiques du développement larvaire (croissance allometrique rapide, restes de membrane natatoire), l'apparition de toutes les structures juvéniles (présence de toutes les écailles, septum nasal, etc.) et la stabilisation de la croissance rélative.

Introduction

The life-history model for fishes proposes a hierarchical system of intervals, consisting of four or five periods depending upon the species: embryo, larva, juvenile, adult, and senescence (Balon 1975, 1981, 1985*a*, 1990). Thresholds that separate periods are more distinct than those separating the shortest saltatory intervals of ontogeny, the steps; the former are easier to distinguish, are of major significance, and are the truly decisive events of ontogeny (Balon 1985*b*). The thresholds between some periods are relatively well documented. For example, the threshold between the embryonic and larval periods is characterised by the onset of exogenous feeding (anlagen of active interaction with other organisms), which is usually accompanied by changes in allometric growth. Similarly, the juvenile to adult transition is also well distinguished, being characterised by the onset of sexual maturity and in many species by reproductive migrations as well.

The transition to juvenile development is described in the life-history model as a metamorphosis in fishes that undergo direct development, such as *Neogobius melanostomus*, *Labeotropheus trewavasae*, *Cyphotilapia frontosa*, or *Latimeria chalumnae* (see Balon 1990). Metamorphosis is a threshold, step, or sometimes longer interval, the juvenile period beginning at a moment when some larval structures still remain and some juvenile–adult structures have yet to appear (the terms indirect development, direct development, and metamorphosis; sensu Balon 1985*b*, 1990). However, the start of the juvenile period in fishes that undergo indirect development is ill defined (Paine and Balon 1984; Flegler-Balon 1989), with juveniles also being referred to as small adults or the definitive phenotype (Balon 1985*b*, 1990; Crawford and Balon 1995). Indeed, since Balon's 1975 article, the ongoing development of the theory of saltatory ontogeny and the hierarchical life-history model of fish (e.g., Balon 1981, 1985*a*, 1990) has not included detailed discussion of the juvenile period. Studies of early fish ontogeny generally do not extend much beyond the

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G.H. Copp.[1] University of Hertfordshire, Division of Environmental Sciences, College Lane, Hatfield, Hertfordshire, AL10 9AB, U.K.
V. Kováč. Comenius University, Faculty of Natural Sciences, Institute of Zoology, Mlynská dolina B2, 842 15 Bratislava, Slovakia.

[1] Author to whom all correspondence should be addressed.
e-mail: g.h.copp@herts.ac.uk

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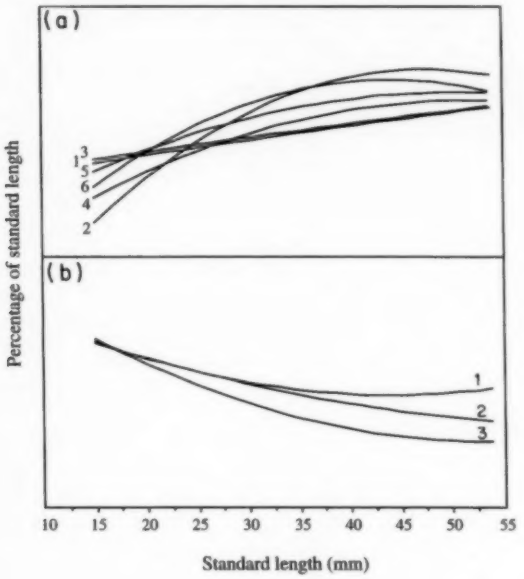
Fig. 1. Patterns in relative growth, as expressed by their polynomial regressions, for mensural characters (in % SL vs. SL) in 0+ roach from the River Great Ouse basin, United Kingdom. The regressions are redrawn as one figure from Kováč and Copp (1996) at different scales without the ordinate values given, as each character had a different range of values. (*a*) 1, pectoral to ventral fin distance; 2, body depth; 3, pectoral fin length; 4, ventral to anal fin distance; 5, body width; 6, ventral fin length. (*b*) 1, preventral distance; 2, head length; 3, eye diameter.



so-called start of the juvenile period (e.g., Balon 1956; Černý 1977; Paine and Balon 1984; Holden and Bruton 1995; Crawford and Balon 1995). This is compounded by the view that juvenile development is beyond the limit of early development (e.g., Krupka 1988); in other words, some ichthyologists consider early development to be over at the end of the larval period. So how early is early? Other reasons aside, the importance of a clear definition of the larval to juvenile transition also becomes obvious when one begins to apply ontogenetic scales for interspecific comparisons of morphological development (Fuiman 1994), to identify ontogenetic shifts in resource use (Juanes et al. 1994), or to reveal evolutionary trajectories of species or genera (Balon 1990).

The comparatively well-studied roach *Rutilus rutilus* (Linnaeus, 1758) is a good example of the ignorance concerning juvenile ontogeny. Until recently (Kováč and Copp 1996), information was available on the morphological development of embryos, larvae (Balon 1956; Černy 1977), and adults (Holčík and Skořepa 1971), but data for juveniles was limited to a few specimens (Balon 1956). Kováč and Copp (1996) found that nine morphological characters in 0+ specimens of roach (15–55 mm standard length (SL)) continued to grow allometrically (either positively or negatively) up to about 40 mm SL; thereafter, relative growth in specimens of 40–55 mm SL was generally isometric and as strongly limited as that previously reported for adults (Fig. 1). These patterns in the relative growth of 0+ roach (Fig. 1) present a dilemma with respect to current interpretations of the life-history model (Flegler-Balon 1989; Balon 1990). The criteria suggested by Balon (1956, 1990) for identifying the transition from the larval to juvenile period in roach (disappearance of most of the finfold, presence of all fin rays, bifurcation and articulation of soft rays, presence of nasal septa, presence of some scales) ignore relative growth, which has yet to stabilise (i.e., isometric growth) in some important characters (Fig. 1) and can have important functional consequences for locomotion, visual acuity, prey handling, and microhabitat use.

Larval metamorphosis in fishes has been described by Geigy and Portmann (1941), strictly on the basis of morphology, using the following three criteria: (*i*) degeneration (histolysis) of some larval characters, (*ii*) new formation of some adult characters, and (*iii*) continuation of common larval–adult characters. However, although metamorphosis occurs in many fishes (Balon 1990), it is more progressive in fishes undergoing indirect development (Flegler-Balon 1989), being more of a transition than metamorphosis, with allometric growth more pronounced in these species than in those of direct development (e.g., Bertin 1958). Thus, in addition to the disappearance of temporary organs and the appearance of some adult structures (e.g., fin ray bifurcation, scales, etc.), the definition of the end of the larval period and the start of the juvenile period should take into greater consideration the patterns in allometric growth (Balon 1975; see also Fig. 1), being that morphological remodelling is central to the concept of larval metamorphosis and juvenile transition in fish (Balon 1990; Crawford and Balon 1995). At the same time, one of the tenants of the theory of saltatory ontogeny is that development consists of more than just morphological changes, ontogeny also being reflected by changes in physiology, behaviour, and niche breath (Balon 1990).

According to the saltatory theory (Balon 1990), ontogeny is a sequence of extended stabilised states (steps) and rapid changes in "integrative actions" (thresholds). Therefore, a threshold in the ontogeny of an individual fish occurs when its form, ability, and activity change concurrently. As body form is not merely a question of external appendages, it remains to be determined whether one of the following events is more important in the transition from larvae to juveniles: (*i*) the more or less simultaneous elimination of some temporary external structures and the appearance of certain adult structures or (*ii*) the stabilisation of allometric growth.

Therefore, the question remains on how to define the transition from larvae to juveniles in fishes that undergo indirect development. Does the transition take place when some temporary organs or structures still remain and some adult structures have yet to appear (Balon 1975), or does it take place when the adult phenotype and a new homeorhetic state in growth variability have been attained?[2] The aim of the present study is to examine whether ontogenetic patterns of relative growth, visual acuity, and microhabitat use in 0+ juvenile roach allow them to be considered small adults (sensu Balon 1985*b*, 1990), i.e., that they possess all the characteristics of

[2] A homeorhetic state of growth variability refers to an overall isometric pattern of growth, though some characters can demonstrate allometric growth during certain shorter intervals of the remaining life history.

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an adult except larger size and sexual maturity. We thus re-evaluate the process of metamorphosis in roach.

Materials and methods

Specimens of 0+ roach were collected using point abundance sampling by electrofishing (Copp and Peňáz 1988) in a habitat typology that was undertaken over 7 weeks in August and September 1990 at 130 sites (rivers, streams, side-channels, backwaters) within the catchment of the River Great Ouse, United Kingdom. For a detailed description of sampling methods, see Copp (1992). Data on the morphological development and the microhabitat use of these specimens were taken from Kováč and Copp (1996) and Copp (1992), respectively. Morphological function (swimming ability: generate lift, reduce drag and energy-inefficient recoil; Webb and Weihs 1986), for the Great Ouse 0+ roach was based on the caudal peduncle depth factor (minimum caudal peduncle depth / maximum body depth) and the body shape factor (SL / maximum body depth), which were calculated as in Webb and Weihs (1986). The ontogenetic development of visual acuity in roach was reassessed using data obtained from Wanzenböck and Schiemer (1989). Additionally, data on the microhabitat use of 0+ roach in the River Great Ouse at Needingworth between May and September 1989 (a temporal study of microhabitat use), and during 3 weeks in the River Danube (along the Hungarian–Slovak border) during August and September 1992 (a habitat typology), were taken from Copp (1996) and Copp et al. (1994), respectively.

Environmental variables were measured qualitatively, semiquantitatively, or quantitatively, then later converted to qualitative categories for analysis (Copp 1992, 1996; Copp et al. 1994). To compare microhabitat use, we recalculated the frequencies of 0+ roach with environmental variables from the data sets reported in the original studies, distinguishing between smaller 0+ specimens (<40 mm SL) and larger 0+ specimens (≥40 mm SL). The environmental variables and their various categories (in parenthesis) used in the typology by Copp (1992) were as follows: channel shape (symmetrical, asymmetrical, trapezoidal), depth (0–10, 10–20, 20–50, 50–100, 100–150, >150 cm), width (0–2, 2–5, 5–10, 10–15, 15–20, >20 m), substrate (leaf bed or organic mud, <0.06 cm; mineral mud or sand and mud, <0.06 cm; sandy silt, sand or silty sand, 0.06–0.2 cm; pebbles and sand or pebbles and mud, 0.2–2.0 cm; gravel, gravel and sand, or gravel and pebbles, 2.0–6.0 cm; rocks, rocks and mud, rocks and sand, or blocks, >6.0 cm), floating vegetation (absent, some, dense), aquatic grasses (absent, some, dense), submerged broad-leafed macrophytes (absent, some, dense), *Myriophyllum* sp. and *Elodea* sp. (absent, some, dense), reeds and phragmites (absent, some, dense), filamentous algae (absent, some, dense), riparian vegetation (absent, some bushes or trees, dense bushes or trees), ligneous debris and roots (absent, some, dense), water velocity (null, weak, medium, strong), water conductivity (<600, 600–899, 900–1199, 1200–1499, 1500–1799, ≥1800 μS), and water temperature (9.0–11.4, 11.5–13.9, 14.0–16.4, 16.5–18.9, 19.0–21.4, ≥21.5°C). The environmental variables used in the typology by Copp et al. (1994) were as follows: distance from bank (0–0.4, 0.5–0.9, 1.0–2.0, 2.1–4.0, 4.1–9.0, >9.0 m), slope of bank (0–0.04, 0.05–0.08, 0.09–0.13, 0.14–0.22, 0.23–0.36, 0.37–0.60, 0.61–1.0), percentage of clay (0, 1–33, 34–66, 67–100), percentage of silt (0, 1–33, 34–66, 67–100), percentage of sand (0, 1–33, 34–66, 67–100), percentage of pebbles (0, 1–33, 34–66, 67–100), percentage of gravel (0, 1–33, 34–66, 67–100), water transparency (0–11, 12–20, 21–33, 34–54, 55–90, 91–148, >148 cm), ligneous structures (0, 1–3, 4–6, >6 structures), macrophytes (0, 1–33, 34–66, 67–100), water velocity (0, ≤5, >5 cm·s^{-1}), and water temperature (≤21, 21.1–23, 23.1–25, 25.1–27, ≥27°C). The variables used in the temporal study by Copp (1996) were the following: situation (channel, littoral interface, weak–medium bank, steep–vertical bank), depth (0–0.2, 0.2–0.5, 0.5–1.0, 1.0–1.5, >1.5 m), substratum (organic matter – leaf bed, mineral mud, sand with silt or mud, alluvia with mud), illumination (sun, shade, overcast), ligneous debris (absent, some, dense), duckweed (absent, some, dense), other macrophytes (absent, present), pondweed (absent, some, dense), filamentous algae (absent, some, dense), and deviation from mean water temperature (<–1.0, –1.0 to 0.51, –0.5 to 0.01, 0 to 0.49, 0.5 to 0.99, ≥1.0°C). Differences in the variables measured and their categories reflect both differences in the general character of the locations studied and progressive improvements in the selection and measurement of variables.

Although not an arbitrary choice (see Kováč and Copp 1996), we recognise that the 40 mm SL cutoff point is a generalisation imposed to facilitate data analysis. The samples by species matrices were converted to absence or presence (0 or 1) to determine the respective frequencies of occurrence and deviations from expected (chi-square, χ^2) with respect to environmental variables as well as to generate environmental profiles of microhabitat use for each species. Using software by Chessel and Dolédec (1991) and Thioulouse (1989), environmental (microhabitat) profiles were calculated and illustrated as the difference between the frequency of a species in the group of samples having a given category of environmental variable and the frequency of that species in all samples containing fish. In these profiles, positive values indicate preference and negative values indicate avoidance, with values approaching |0.5| indicating χ^2 deviations from expected (Chessel and Dolédec 1991). Because the association analysis was undertaken on the reduced matrices (i.e., all void samples eliminated), calculations of χ^2 probabilities may be considered as conservative. Changes in the microhabitats used by the <40 and ≥40 mm SL roach were identified using the Mann–Whitney U test (Sokal and Rohlf 1966), where for a given variable the raw qualitative categorical data (1, 2, 3, 4, etc.) for the two groups of young roach were compared.

Results

The roach may be considered amongst the thunniform (cruiser) type of fish morphology for which body form factors are thought to attain adult values by a total length of about 6 times the length at hatching (Webb and Weihs 1986). For Great Ouse 0+ roach, the 15–40 mm SL interval ends at 6.9 times the SL at hatching (Fig. 2), with roach of ≥40 mm SL possessing caudal peduncle depth factors (ratio between the minimum depth of the caudal peduncle and the maximum body depth) similar to that of adults (Fig. 2), though still significantly different (Mann–Whitney U test, $p = 0.0001$). However, contrary to the pattern reported by Webb and Weihs (1986) for thunniform fishes, the body shape factor (ratio between the standard length and the maximum body depth) continued to increase beyond 6 times the length at hatching, levelling off somewhere between 9 and 20 times (Fig. 2). Thus, although 0+ roach of ≥40 mm SL are able to generate lift, reduce drag, and reduce energy-inefficient recoil movements similar to adults (see Webb and Weihs 1986), they do not yet possess as streamlined a body shape as adults.

The reactive distance of 0+ roach to prey stabilises at 35–45 mm SL (Fig. 3). The increase in reactive distance with increasing size is best fit by a polynomial equation (greater r^2 value), contrary to Wanzenböck and Schiemer's (1989) linear model, with values levelling off at 35–45 mm SL. Similarly, the minimum separable angle (in minutes) decreases exponentially until 35–45 mm SL, where it appears to level off at about 100 min (Fig. 3), decreasing further to about 30 min in specimens of 70–90 mm SL (J. Wanzenböck, Institute of Limnology, Mondsee, Austria, personal communication). These two aspects of visual acuity reflect the growth of the eye, which is

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Fig. 2. Two body form factors related to the functional locomotor morphology of fishes: body shape factor (SL / maximum body depth) and caudal peduncle depth factor (minimum caudal peduncle depth / maximum body depth). Both factors are plotted against the ratio between SL and length at hatching, which is approximately 6 mm SL in roach of the River Great Ouse (G.H. Copp, unpublished data). The values for 0+ specimens are from Kováč and Copp (1996), whereas those of adult roach were taken from Holčík and Skořepa (1971) for Slapy Reservoir (Czech Republic).

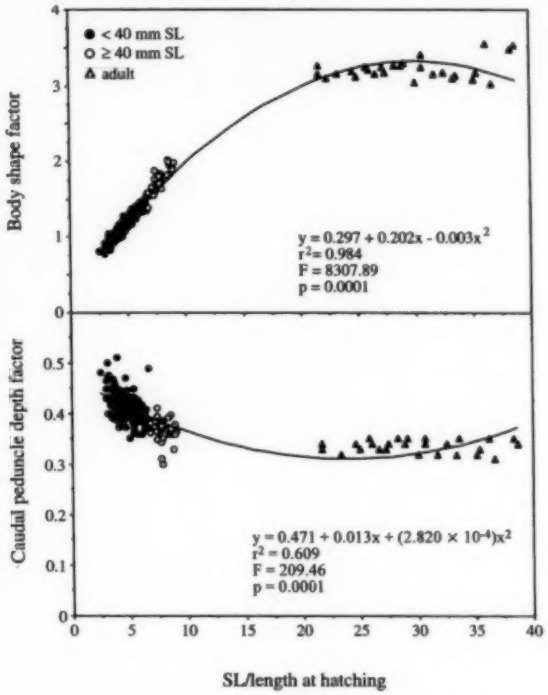
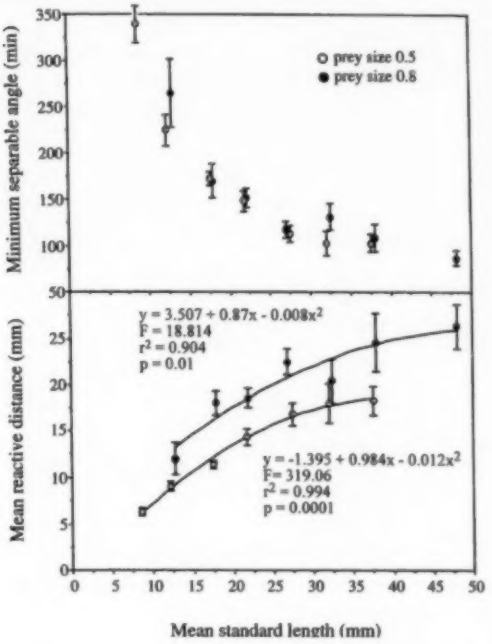

● < 40 mm SL
○ ≥ 40 mm SL
△ adult
Body shape factor
y = 0.297 + 0.202x - 0.003x²
r² = 0.984
F = 8307.89
p = 0.0001
Caudal peduncle depth factor
y = 0.471 + 0.013x + (2.820 × 10⁻⁴)x²
r² = 0.609
F = 209.46
p = 0.0001
SL/length at hatching

Fig. 3. The minimum separable angle (in minutes) and the mean reactive distance (in millimetres) to prey (size 0.5 and 0.8 mm) of 0+ roach with increasing SL (redrawn with data from Wanzenböck and Schiemer 1989).


○ prey size 0.5
● prey size 0.8
Minimum separable angle (min)
y = 3.507 + 0.87x - 0.008x²
F = 18.814
r² = 0.904
p = 0.01
y = -1.395 + 0.984x - 0.012x²
F = 319.06
r² = 0.994
p = 0.0001
Mean reactive distance (mm)
Mean standard length (mm)

negatively allometric up to 40–50 mm SL (Fig. 1), as well as the development of signal integration; the temporary stabilisation at 35–45 mm SL of both reactive distance and visual angle suggests the attainment of a threshold in development, with the adult or definitive level of visual development being achieved later.

Recalculation of species habitat frequencies revealed differences in all cases between the microhabitat use of <40 mm and ≥40 mm SL roach (Mann–Whitney, $p = 0.05$); the differences reflect a move from the littoral zone (as <40 mm SL roach) to the pelagic zone (as ≥40 mm SL roach). In the Great Ouse basin (Fig. 4), 0+ roach of ≥40 mm SL differed in microhabitat use from those of <40 mm SL by avoidance of floating macrophytes and riparian cover, a much reduced preference for marginal vegetation (reeds, phragmites), and an indifference to water velocity. The differences in water temperature preferences between younger and older roach probably reflect the gradual decrease in ambient water temperature during late summer and early autumn. In the typological study on the River Danube, roach of ≥40 mm SL differed in microhabitat use from <40 mm roach with respect to substrate; those <40 mm SL preferred clay, silt, and sand, whereas those of ≥40 mm SL preferred pebbles and gravel (Fig. 4). The smaller roach preferred turbid waters with much submerged vegetation, whereas the larger roach preferred transparent waters with little submerged vegetation. The larger roach also became relatively indifferent to water velocity, the weak avoidances not being significant (χ^2; Fig. 4). In the temporal study of roach development in the River Great Ouse at Needingworth, the <40 mm SL roach were associated with ligneous debris and the river's littoral interface, avoiding dense pond weed, filamentous algae, and the main channel (Fig. 4), whereas the ≥40 mm SL roach were generally indifferent to all variables, with very weak preferences for open water areas up to 0.5 m deep.

Discussion

Although our results shed some light on a poorly understood part of the life history of fishes, the larval to juvenile transition remains poorly defined. In a number of species, remnants of larval characteristics, such as the fin fold (Copp and Peňáz 1988), absence or incomplete scale cover (Balon 1975), and persistent notochord in the caudal fin (Kováč 1995), are commonly reported in specimens otherwise considered as 0+ juveniles (e.g., in which most fin rays are bifurcated, the fin fold has differentiated, and the nasal septa have appeared). In roach, this phenomenon can be seen as a break in the logarithmic relationship between length and weight (Balon 1985*b*; Copp and Peňáz 1988, Mann 1996). However, completion of the remodelling process (Balon 1990) is of equal importance

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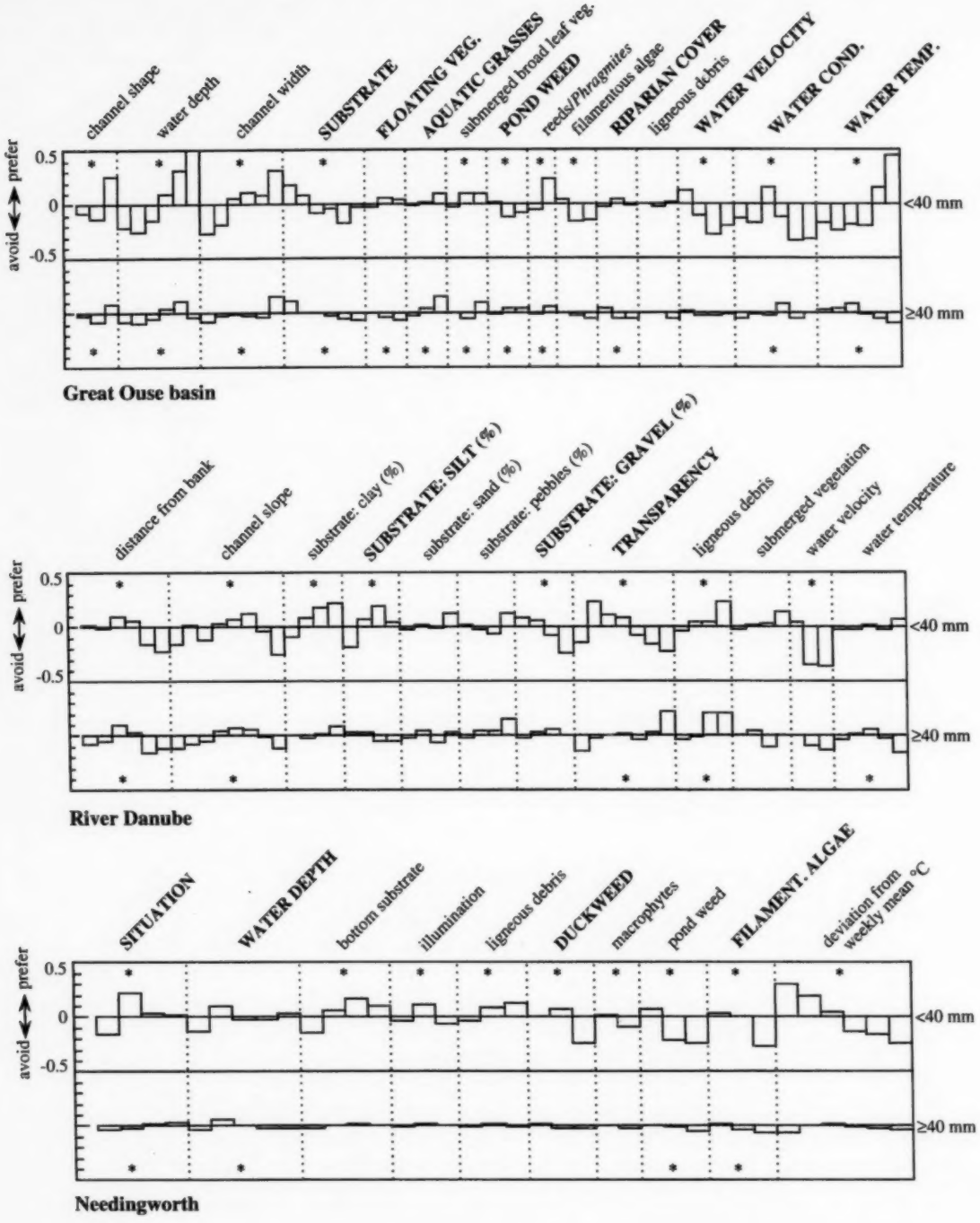

in defining the larval to juvenile transition as changes to external structures, shifts in niche, and changes in behaviour (Balon 1985*b*).

The importance of defining the remodelling process is clear in recent work on the bluefin killifish, *Lucania goodei*, in which Crawford and Balon (1995) used external patterns in relative growth to complement (even corroborate) patterns in internal development (e.g., skeletal calcification, organogenesis). However, because their study did not extend into the so-called juvenile period beyond about 4 days, it remains

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Fig. 4. Microhabitat profiles and χ^2 deviations from expected for <40 and ≥40 mm SL roach recalculated from data for the Great Ouse typology (Copp 1992), the River Danube typology (Copp et al. 1994), and the temporal study in the River Great Ouse at Needingworth (Copp 1995). Each histogram represents the difference between the frequency of that size-class in the group of point samples having that category of environmental variable (see Materials and methods for categories) and the frequency of that size-class in the samples. Significant χ^2 associations ($p < 0.05$) between size-classes and variables are indicated with an asterisk, and between size-classes and individual categories by histogram values approaching |0.5|. Where significant differences (Mann–Whitney U test, $p \leq 0.05$) were found between the <40 mm and the ≥40 mm SL roach with respect to a given environmental variable, that variable is given in boldface uppercase letters (two nearly significant differences were pebble substrate and water temperature, $p = 0.06$).

unclear whether the juvenile period had in fact been achieved; a number of important relative growth characters still differed between adults and the so-called juveniles (snout length, eye diameter, head length, caudal peduncle depth), and calcification remained incomplete (Crawford and Balon 1995).

The continued allometric growth of many mensural characters in smaller 0+ roach (Fig. 1) suggests that remodelling is incomplete until relative growth has stabilised, accompanied by the disappearance of all remnants of larval development (e.g., fin fold, notochord) and the presence of completed juvenile–adult structures (e.g., fin ray bifurcation, nasal septa, scale cover). This perspective is consistent with the idea that the process of remodelling terminates the larval period, i.e., the process must be completed for a larva to be considered as having breached the larval to juvenile transition (Balon 1990) and become a juvenile. In other words, 0+ roach of 15–40 mm SL (Fig. 1) were in the midst of completing larval metamorphosis, and had not yet achieved the definitive or adult form (sensu Balon 1990). Additional support for this interpretation, that roach of approximately 15–40 mm SL are in the last step of the larval period, is found in the functional locomotor morphology (Fig. 2), the development of visual acuity (Fig. 3), and the microhabitat shift from the littoral to the pelagic (Fig. 4).

The 15–40 mm SL interval could alternatively be viewed as the first stabilised state (step) of the juvenile period (sensu Balon 1975) in which the "cells and tissues differentiated and structures (organs) grow at various rates [our Fig. 1], as if accumulated and canalized in preparation for the next, more specialised state" (Balon 1990), i.e., the second juvenile step. Changes in the logarithmic relationship between length and weight (Copp and Peňáz 1988) would support this viewpoint. In one of the few studies to address the ecomorphology of young juveniles, the transition to the juvenile period in rainbow darter, *Etheostoma caeruleum*, was defined by formation of a jointed vertebral column, and separated, flexible median fins, which were viewed as adult characteristics important to locomotion (Paine and Balon 1984). However, consistent with Balon (1975), another adult feature, scale cover, did not appear until later, when its appearance was used to designate the second juvenile step. By contrast, in roach (Balon 1956) and in barbel, *Barbus barbus* (Krupka 1988), the onset of scale cover was a major attribute used to designate the beginning of the juvenile period. In roach (Balon 1956), scales begin to appear at 16.5–20.0 mm SL, with the second juvenile step beginning once complete scale cover has been achieved (at 22 mm SL; on the basis of one specimen only). The onset of scale cover varies greatly between species and thus is probably less valuable an attribute than completion of scale cover in defining (or contributing to the definition of) a threshold.

In conclusion, the definition of transition to the juvenile period in fishes with indirect development is still rather arbitrary (Paine and Balon 1984; Flegler-Balon 1989), which is not surprising in view of the lack of information on larval and juvenile development. The criteria used to define juvenile transition for the rainbow darter (Paine and Balon 1984), the barbel (Krupka 1988), the bluefin killifish (Crawford and Balon 1995), and probably 0+ juvenile roach (Balon 1956) would appear not to support the view that juveniles are little adults (Balon 1984, 1985*b*, 1990), as one would expect a small adult to possess all the characteristics of an adult except larger size and sexual maturity. Our results could be used to support either of the interpretations given above; however, we feel that to designate roach of 15–40 mm SL as juveniles (sensu Balon 1975) is at variance with the underlying principles of the theory of saltatory ontogeny (Balon 1990) and with the available evidence (Figs. 1–4). We propose this interval to be the final, and previously unrecognised, step of the larval period. We suggest that further extensive study of juvenile development, preferably as part of investigations addressing a species' entire ontogeny, is required to resolve this question. We note, however, that designation of the threshold will be complicated by the fact that descriptions of ontogeny based on samples of different individuals will inherently reflect the noise of individual variation rather than the true individual ontogeny (Balon 1990).

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Responses of eastern mosquitofish (*Gambusia holbrooki*) to acute salinity stress: do family effects obscure inbreeding and genotype effects?

Karen L. Kandl and Andrew J. Thompson

Abstract: Eastern mosquitofish (*Gambusia holbrooki*) having inbreeding coefficients of 0, 0.125, and 0.25 were exposed to a salinity stress of 14.34 parts per thousand (ppt) for 96 h, and their survival times were determined. Statistical models were fitted to the failure-time data to assess differences between the survival functions based on inbreeding coefficient, family, and genotype of the fish. Predicted median survival times were determined for inbreeding classes, families within inbreeding classes, classes based on number of heterozygous loci, and genotypes at single loci. Family significantly affected survival of individuals in all analyses, regardless of the inbreeding coefficient of the individual. The inbreeding coefficient of an individual did not statistically affect survival. The number of heterozygous loci per individual significantly affected survival time but only when family as well as number of heterozygous loci per individual were included as independent variables in the analysis. Genotypes of two loci, *m*-isocitrate dehydrogenase A and glucose phosphate isomerase B, also significantly affected survival. Our study demonstrates that family can account for a greater proportion of the variance in survival under environmental stress than either inbreeding coefficient or number of heterozygous loci. Family effects, in addition to inbreeding, should be considered in conservation management plans.

Résumé : On a exposé la gambusie (*Gambusia holbrooki*) possédant des coefficients de consanguinité de 0, 0,125 et 0,25 pendant 96 h à un milieu dont la salinité était de 14,34 ppt, puis on a déterminé son temps de survie. On a appliqué des modèles statistiques aux données échec-temps en vue d'évaluer les différences entre les fonctions de survie basées sur le coefficient de consanguinité, la famille et le génotype du poisson. On a déterminé la valeur médiane des temps de survie prévus pour les classes de consanguinité, les familles à l'intérieur de ces classes, les classes basées sur le nombre de locus hétérozygotes et les génotypes à des locus seuls. Dans toutes les analyses, la famille influait de façon significative sur la survie des individus, indépendamment du coefficient de consanguinité de l'individu. Le coefficient de consanguinité d'un individu n'influait pas statistiquement sur la survie. Le nombre de locus hétérozygotes par individu influait beaucoup sur le temps de survie, mais seulement lorsque la famille ainsi que le nombre de locus hétérozygotes par individu était inclus comme variables indépendantes dans l'analyse. Les génotypes de deux locus, la *m*-isocitrate-déshydrogénase A et la glucose-phosphate-isomérase B, influaient aussi considérablement sur la survie. Notre étude montre que la famille permet d'expliquer une plus grande proportion de la variance dans la survie en présence d'un stress environnemental, que le coefficient de consanguinité ou le nombre de locus hétérozygotes. En plus des effets de la consanguinité, il y aurait lieu de tenir compte des effets de la famille lors de l'élaboration de plans de gestion à des fins de conservation.
[Traduit par la Rédaction]

Introduction

For the past two decades, conservation genetics has focused largely on the effects of inbreeding and levels of genetic variation on individual survival. Other genetic effects, such as family effects, may be important to an organism's fitness, especially in polluted or degraded environments. Family effects are fitness responses of individuals that depend upon the unique genetic background of the lineage or family. Family effects have been found to account for differences in body size in frogs (Travis 1980, 1981), body shape in fish (Wimberger 1992), and individual growth rate in frogs (Travis 1980, 1981). Despite the fact that family effects have been documented in the ecological and genetic literature, they have commonly been ignored in conservation genetics and inbreeding studies.

Breeding programs, including those for aquatic organisms, generally recognize the importance of genetic variation to populations (Waples 1994; Hedrick and Hedgecock 1994; DeMarais and Minckley 1993). However, many studies and breeding programs still place heavy emphasis on inbreeding effects and inbreeding coefficients without regard for other genetic effects (Kincaid 1983; Mallet and Haley 1983; Johnsson and Abrahams 1991). Inbreeding depression is a serious concern, but its importance relative to family effects may be small. Where researchers have studied both inbreeding and family effects,

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K.L. Kandl.[1] Savannah River Ecology Laboratory, Drawer E, Aiken, SC 29802, U.S.A., and Department of Zoology and Institute of Ecology, University of Georgia, Athens, GA 30602, U.S.A.
A.J. Thompson. Savannah River Ecology Laboratory, Drawer E, Aiken, SC 29802, U.S.A.

[1] Author to whom all correspondence should be addressed. e-mail: kandl@srel.edu

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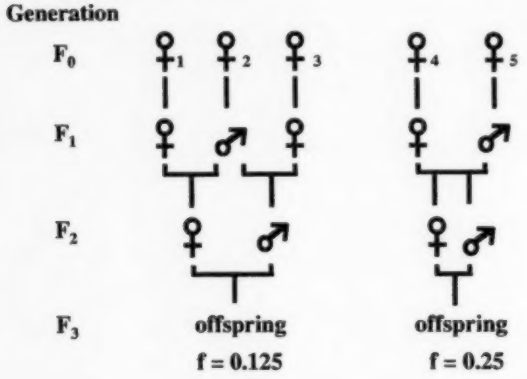
Fig. 1. Mating scheme used to obtain mosquitofish with inbreeding coefficients of either $f = 0.25$ or $f = 0.125$ in the F_3 generation. Females in the F_0 generation were already pregnant when collected from the field.



family effects have been found to be important to fitness. In an inbreeding study, Wright (1977) found that differentiation among lineages owing to inbreeding significantly affected fitness-related traits in guinea-pigs (*Cavia porcellus*), including size of litter, litters per year, young raised per year, and juvenile weight gain. Wolfe (1993) found that maternal effects masked inbreeding effects on seed size and seedling growth in *Hydrophyllum appendiculatum*, a biennial plant. Pray et al. (1994) found that inbred lineages of red flour beetles (*Tribolium castaneum*) had differential survival that could be attributed to family effects. When family effects are ignored, two problems may result. First, failure to recognize family effects may result in an error in the estimation of inbreeding depression, which is the reduction in fecundity, survivorship, or other fitness-related traits owing to inbreeding. Second, family effects on fitness-related traits may outweigh effects of inbreeding depression so that the relative importance of inbreeding is miscalculated.

The environment in which an organism lives can determine, in part, the relative importance of family, inbreeding, and other genotype effects to fitness-related traits. However, the environment is often not taken into account in inbreeding studies. Inbreeding depression is consistently demonstrated only in zoo or captive populations, which live in relatively benign environments (Ralls and Ballou 1983; Templeton and Read 1983; Wildt et al. 1987; Lacy et al. 1993). Inbreeding depression is dependent on the environment (Lacy et al. 1993; Pray et al. 1994).

Likewise, negligence of the environment may obscure genetic effects other than inbreeding effects. Several studies have shown that the effects of amount of genetic variation are more apparent in stressful environments than in benign environments. For example, heterozygous eastern mosquitofish (*Gambusia holbrooki*) had a greater time to death (TTD) during acute exposure to inorganic mercury than did their more homozygous counterparts (Diamond et al. 1989; Newman et al. 1989). Teska et al. (1990) demonstrated that heterozygous old-field mice (*Peromyscus polionotus*) used food more efficiently and maintained body weight under dietary stress better than less heterozygous mice.

The expression of family effects is also dependent on the environment. McAlpine (1992) showed that in a predator-free environment, family affected survival and tail and limb injury of green tree frogs (*Hyla cinerea*) but did not affect these traits in the presence of predators. Several studies have shown that the average fitness of individuals within families depends on the environment (Miller 1994; Pray et al. 1994). When these average fitness values are examined in different environments, they change as the environment changes. For example, Pray et al. (1994) found that the rank order of average fitnesses for families of red flour beetle lineages changed depending on the environment of the beetles.

We report the results of an experimental study of survival in salinity stress using inbred eastern mosquitofish. First, we examined how family or lineage affected survival of mosquitofish exposed to salinity stress using 12 families for each of three inbreeding classes. Second, we tested whether salinity stress had a greater impact on inbred than on outbred fish. Third, we examined the relationship between short-term survival and both number of heterozygous loci and single-locus genotypes in a high salt environment. We predicted that family would differentially affect survival of mosquitofish under salinity stress. We also predicted that survival would decrease as inbreeding increased and number of heterozygous loci per individual decreased.

Materials and methods

Fish collection and breeding

Pregnant mosquitofish (F_0 generation) were collected from Little Hell Landing on the Savannah River in Barnwell County, South Carolina, in July 1992. This landing is on an oxbow lake surrounded by swamp. F_0 females were assumed to originate from a large panmictic population and to be unrelated to each other.

Offspring of F_0 females (F_1 generation) were born in early August 1992 and raised to maturity (September 1992) in separate containers in a recirculating system. F_1 males were randomly mated with either one or two virgin F_1 females, other than their siblings, in October 1992.

Offspring of F_1 matings (F_2 generation) were born from late November through December. Juveniles from each family were kept in the same container until they began to mature. Males were separated from their siblings when their gonopodia began to form.

Virgin F_2 males and females were mated in March and April 1993 to obtain offspring (F_3 generation). Two inbreeding classes, $f = 0.125$ and $f = 0.25$, and a control, $f = 0$, were used for this experiment. We chose these levels of inbreeding because they are relatively high for natural populations but are not unrealistic. First, F_3 individuals with inbreeding coefficients of $f = 0.125$ were obtained by mating F_2 offspring of F_1 females that had been mated to the same male (paternal half-sibs) (Fig. 1). Second, F_3 individuals with inbreeding coefficients of $f = 0.25$ were obtained by mating F_2 full siblings (Fig. 1). Third, control mosquitofish ($f = 0$) were offspring of pregnant females collected from a presumably panmictic population of the Savannah River near Steel Creek delta in Barnwell County, South Carolina. We attempted to collect control mosquitofish where F_0 females had been collected, but because of high flooding of the Savannah River in spring 1993, the original population was no longer present. Steel Creek delta is 35 km upriver from Little Hell Landing. These $f = 0$ offspring were born in early June 1993 and raised in family groups. Each inbreeding class was represented by 12 families (Table 1).

Experiment

In July 1993 we performed an acute salinity stress experiment with a

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TTD response similar to that used by Newman and Aplin (1992). This was performed at the Savannah River Ecology Laboratory near Aiken, South Caroloina. Two 230-L recirculating water tanks were used, one for the control treatment and the other for the experimental treatment. Each tank system consisted of a steel stand that held two separate plate-glass trays. Each tray contained clear plastic containers. The control system held a total of 96 containers, and the experimental system held a total of 112 containers. Each plate-glass tray with its containers was covered by a perforated plexiglass dispersion tray that provided a constant flow of water into each container. Small holes were drilled near the bottom on the downstream side of each container so that the excess water flowed into the plate-glass tray. The water then flowed from the plate-glass tray through a filter and was diffused into a storage tank by strips of plate glass and foam filters. A pump circulated the water from the storage tank, through a chiller, and back up onto the dispersion trays. This recirculating system kept water at a constant temperature and salinity in the tanks. Water used for this experiment was pumped from Upper Three Runs Creek, an uncontaminated creek on the Savannah River site. The water was allowed to sit for approximately 1 day so that sediments settled out.

Seventy-nine fish were placed in the control tank (stream water with no additional sodium chloride added); 279 fish were placed in the experimental tank. Family groups of mosquitofish were randomly assigned to the cells within the control and experimental tanks. Up to four fish were placed into each cell, depending on the size of the family. There was no evidence of overcrowding, such as death or cannibalism, in any of the cells. Fish were acclimated to the tanks for 3 days before starting the experiment. They were fed ad libitum during this acclimation time but not fed during the experiment.

The lethal concentration of NaCl expected to kill 80% of the fish (LC_{80}) was estimated from Newman and Aplin (1992) to be 14.23 parts per thousand (ppt) . This concentration was also tested empirically with a small number of fish to ensure that it would kill 80% of the fish over 4 days. At the onset of the experiment, 3273 g of food-grade NaCl was added to the experimental tank over a 20-min period. Shortly thereafter, the conductivity of the water in the tanks was measured using a hydrolab surveyor II. The NaCl concentration in the experimental tanks was determined to be 14.34 ppt. Water samples were taken at the beginning and end of the experiment from both the control and experimental tanks to determine the concentration of sodium and other ions.

The experiment was carried out for 96 h. Control and experimental tanks were checked for dead fish every 4 h. Dead fish were removed, measured to the nearest millimetre (standard length), weighed to the nearest 0.01 g (wet weight), and frozen on dry ice until transferred to a –70°C freezer for storage. When possible the sex of the fish was noted, although most of the fish were juveniles of undetermined sex.

Table 1. Sample sizes for inbreeding classes (N_c) and families (N_f), predicted median survival times (MTTD) for families of mosquitofish in acute salinity stress, and 95% confidence intervals of the MTTD.

Inbreeding class	N_c	Family	N_f	MTTD	95% CI
f=0	110	1	6	46.47	36.33–62.90
		2	23	45.10	39.61–52.12
		3	10	30.54	27.25–35.33
		4	7	30.57	26.76–36.53
		5	11	43.63	36.54–53.75
		6	15	51.78	43.42–63.14
		7	12	41.41	35.22–50.12
		8	9	29.42	26.37–34.03
		9	3	29.16	24.63–38.13
		10	3	30.86	25.48–41.49
		11	7	30.38	39.43–67.51
		12	4	29.73	25.39–37.58
f=0.125	62	88	3	32.43	26.28–44.61
		89	1	24.00	21.23–33.06
		95	11	44.34	37.04–54.77
		98	3	41.53	30.88–62.63
		99	11	41.93	35.35–51.33
		100	5	41.39	33.14–54.82
		101	6	42.88	34.12–57.08
		102	3	39.73	29.97–59.06
		103	3	62.77	41.61–104.71
		104	7	50.51	40.01–66.53
		106	6	32.34	27.62–40.01
		110	1	61.73	37.32–120.55
f=0.25	101	87	12	60.79	49.62–74.27
		90	6	96.62	72.58–131.66
		91	22	41.69	36.85–47.91
		92	9	35.52	30.46–43.03
		96	6	31.31	26.98–38.34
		97	2	44.00	30.40–75.39
		109	5	47.45	36.85–64.73
		113	3	43.39	31.82–66.31
		114	3	29.16	24.63–38.13
		115	10	34.06	29.67–40.44
		116	2	40.78	29.01–67.97
		117	9	29.69	26.53–34.38

Note: See text for a description of how MTTD values were calculated.
f = inbreeding coefficient.

Electrophoresis

Horizontal starch gel electrophoresis was performed on all fish used in this experiment. The following loci were examined: adenosine deaminase (*ADA**) (EC 3.5.4.4), glucose-phosphate isomerase A and B (*GPI-A**, *GPI-B**) (EC 5.3.1.9), *m*- and *s*-isocitrate dehydrogenase A (*mIDHP-A**, *sIDHP-A**) (EC 1.1.1.42), malate dehydrogenase A and B (*sMDH-A**, *sMDH-B**) (EC 1.1.1.37), mannose-6-phosphate isomerase (*MPI**) (EC 5.3.1.8), and phosphoglucomutase 1, 2, and 3 (*PGM-1**, *PGM-2**, *PGM-3**) (EC 5.4.2.2). *GPI-A**, *GPI-B**, *ADA**, *MPI**, *PGM-2**, *sMDH-A**, and *sMDH-B** were run on gels with Tris–citrate buffer, pH 8.0 (Selander et al. 1971), and *mIDHP-A**, *sIDHP-A**, *PGM-1**, and *PGM-3** were run on gels with Tris–citrate–EDTA buffer, pH 7.1–7.2 (Ayala et al. 1972). Results are reported for only the polymorphic loci.

Data analysis

Genetic analyses to determine the fit of observed data to Hardy–Weinberg equilibrium expectations and to determine average heterozygosity per individual estimated by direct count ($\bar{H}$) were performed using BIOSYS-1 software (Swofford and Selander 1981). All other analyses were performed using the SAS statistical package (SAS Institute Inc. 1985). Survival analyses of the data were performed using the LIFEREG and LIFETEST procedures in SAS. LIFEREG is a parametric survival analysis procedure that can be used for right-censored data to test for differences in response variables owing to certain independent variables. Simply stated, LIFEREG fits a statistical model to failure-time data and uses the model to estimate differences between survival functions of groups of individuals owing to one or more variables. It then assesses whether these differences are statistically significant (Dixon and Newman 1991). The model for the response variable includes a function that describes how groups, defined by independent variables, differ from one another and an error term with a user-specified distribution (Dixon and Newman 1991). In the LIFEREG procedure, one of several underlying distributions can be chosen for the error term. In this study, the

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Table 2. Sumary of results of survival analyses. Models and the independent variables used to analyze survival data are listed.

Model	Independent variable	df	χ^2	$P < \chi^2$
A	Family	35	188.9316	0.0001
	f=0.125 vs. f=0	1	1.9277	0.1650
	f=0.25 vs. f=0	1	2.8138	0.0935
	f=0.25 vs. f={0, 0.125}	1	0.0046	0.9462
B	Heterozygosity	3	8.7769	0.0324
	Family	35	189.1812	0.0001
C	Heterozygosity	3	6.1912	0.1027
D	*GPI-B** genotype	2	18.0286	0.0001
E	*mIDHP-A** genotype	4	17.0297	0.0019
F	*sIDHP-A** genotype	1	3.2272	0.0724
G	*PGM-1** genotype	2	0.2901	0.8650
H	*PGM-2** genotype	2	0.9105	0.6343
I	*MPI** genotype	2	3.8557	0.1455

Note: The dependent variable was hours = 20 for all cases. Models A and B were tested with LIFEREG and the remaining were tested with LIFETEST in the SAS statistical package (SAS Institute Inc. 1985).

LIFEREG procedure was used to test for significant differences in TTD between fish with different inbreeding coefficients and families. We chose a lognormal error distribution by comparing the maximum log likelihood values of the models created with various distributions. For models generated in the LIFEREG procedure, a better fit of the data to a lognormal distribution was achieved when the dependent variable was transformed by subtracting 20 h from all observations. Only one fish died within the first 20 h; for statistical analysis, its TTD was reassigned as 4 h since nonpositive values could not be used in the analysis. This transformation of the response variable was used in the LIFETEST procedure as well.

Models A and B (Table 2) were produced in the LIFEREG procedure. The significance of inbreeding coefficient and family was determined in model A using a user-generated model. The user-generated model was required to equally weight all families used in the experiment and set up contrasts to test the following null hypotheses: (*i*) the survival curves for $f = 0$ and $f = 0.125$ were not significantly different, (*ii*) the survival curves for $f = 0$ and $f = 0.25$ were not significantly different, and (*iii*) the survival curve for $f = 0.25$ was not significantly different from the survival curves for both $f = 0$ and $f = 0.125$ (Table 2, model A).

For these models the predicted median TTD (MTTD) for each family was calculated using the following equation: $\text{MTTD} = \exp(\mu + \beta_{\text{Fam}}) + 20$, where μ is the model intercept, a measure of average survival time of a reference family, and β_{Fam} is a parameter estimate for the effect of a particular family. To determine the MTTD, 20 h was added back to the equation. A more general model may be found in Dixon and Newman (1991).

Effects of allozymes on survival were tested with a nonparametric life table procedure, the LIFETEST procedure in SAS. The LIFEREG procedure was not appropriate for allozyme data because the residuals from lognormal distributions were not normally distributed. The LIFETEST procedure compares survival curves to determine whether two or more samples could have arisen from identical survival functions (SAS Institute Inc. 1985). The LIFETEST procedure calculates two tests of this; the log-rank test and the Gehan–Wilcoxon test (Dixon and Newman 1991). All results given are from the log-rank test for homogeneity of survival functions. The log-rank test places greater emphasis on later survival times. For genotypes of loci that were found to be significant to survival time, the LIFEREG procedure was used to determine the predicted MTTD for each of the single-locus genotypes. A Bonferroni test was used to correct for multiple statistic tests.

The significance of the number of heterozygous loci per individual to survival was also tested using both the LIFEREG and LIFETEST procedures. Although the residuals were not normally distributed, the LIFEREG procedure was used in model B (Table 2) so that the independent variable, family, could be incorporated into the model. The LIFETEST procedure was also used to determine MTTD of individuals in each class of number of heterozygous loci. Individuals were heterozygous at zero, one, two, or three loci, yielding four classes.

A χ^2 test based on the log-likelihood values was used to determine whether additional terms significantly increased the fit of the models. Homoscedasticity of variances was tested using Levene's test in an analysis of variance. Significance levels of $\alpha = 0.05$ were used throughout the data analysis unless otherwise stated.

To determine if the manner in which genotypes were distributed among families was confounding family effects, we performed two statistical tests. First, we included *mIDHP-A** and *GPI-B** loci as the only independent variables in a model in the LIFEREG procedure. We then added the variable family to determine if the fit of the model significantly improved. We also examined the correlation between survival of members of a particular family given that family, and survival of members of a particular family given the *mIDHP-A** and *GPI-B** genotypes of individuals within that family. If family effects were due to single locus effects, then one would expect a high correlation between the two terms above. We also used the same procedure to determine if family effects were confounded by the effect of multilocus heterozygosity. Specifically, we determined if there was a correlation between survival of members of a particular family given that family, and survival of members of a family given the number of heterozygous loci of individuals within that family.

Table 3. Allele frequencies for all polymorphic loci in the control and inbreeding groups.

		Inbreeding coefficient		
Locus	Allele	$f = 0$	$f = 0.125$	$f = 0.25$
*GPI-A**	100	1.000	1.000	0.995
	84	0.000	0.000	0.005
*GPI-B**	100	0.995	1.000	0.950
	66	0.005	0.000	0.050
*mIDHP-A**	134	0.382	0.491	0.600
	116	0.020	0.000	0.016
	100	0.598	0.509	0.384
*sIDHP-A**	161	0.000	0.056	0.000
	100	1.000	0.944	1.000
*MPI**	109	0.073	0.089	0.010
	100	0.927	0.911	0.990
*PGM-1**	100	0.745	0.839	0.906
	86	0.255	0.161	0.094
*PGM-2**	181	0.565	0.419	0.356
	100	0.435	0.581	0.644

Results

Genetic data

All of the genotypes in the inbreeding classes were found to be in Hardy–Weinberg equilibrium with the exception of *PGM-2**, which had an excess of heterozygotes in all three of the inbreeding classes (Table 3). There was a trend of decreasing $\overline{H}$ as inbreeding coefficient increased: $\overline{H} = 1.191$ for $f = 0$, $\overline{H} = 1.127$ for $f = 0.125$, and $\overline{H} = 1.050$ for $f = 0.25$. There was,

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Fig. 2. Survival curves for a control group of mosquitofish and the three groups of mosquitofish, $f = 0$, $f = 0.125$, and $f = 0.25$. Control mosquitofish were maintained in a tank lacking sodium chloride but similar to that of the experimental tank for the duration of the experiment. Survival data were taken over 96 h.

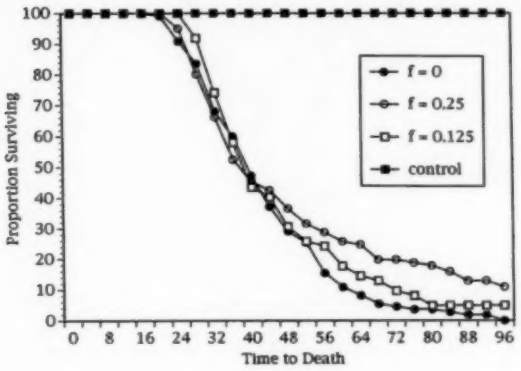
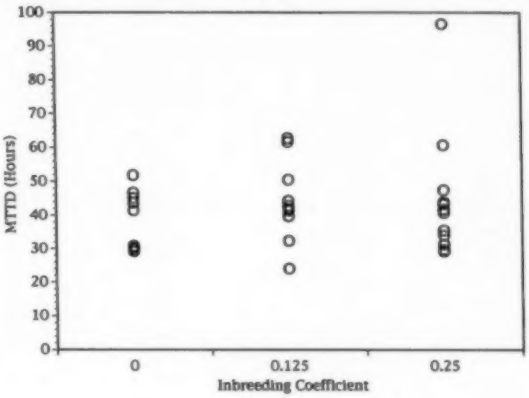


Fig. 3. Predicted MTTDs for each family within inbreeding groups, $f = 0$, $f = 0.125$, and $f = 0.25$. Predicted MTTDs for families are represented by circles. Each inbreeding class contains 12 families; however, predicted MTTDs may be similar so that dots are overlapping.



however, no significant difference in $\bar{H}$ among the three inbreeding classes ($P = 0.40$).

Survival analysis

Five percent (14 of 279) of the fish in the experimental tank survived, whereas all of the fish in the control tank survived, regardless of inbreeding coefficient (Fig. 2). Therefore, control fish were excluded from further statistical analyses. In preliminary survival analyses, weight of the fish was initially included as a covariate; however, weight was not significant and was excluded from the final analyses.

MTTDs varied greatly among families (Table 1). In model A (Table 2), family significantly affected survival in salinity stress ($P = 0.0001$), regardless of the inbreeding coefficient of the family. Because of the design of this experiment, family is nested within inbreeding class. When inbreeding groups were analyzed separately using three separate models, family still significantly affected survival in each of the three models ($P = 0.0001$ for $f = 0$; $P = 0.003$ for $f = 0.125$; and $P = 0.0001$ for $f = 0.25$). Although the variance in survival time increased as the inbreeding coefficient increased (Fig. 3), the variance among families within inbreeding groups was not significantly different using Levene's test for homoscedasticity ($P = 0.640$).

The predicted MTTDs for the three inbreeding classes were 36.22 h for $f = 0$, 39.84 h for $f = 0.125$, and 40.22 h for $f = 0.25$. A test of whether survival time of at least one of the inbreeding classes was different from the others was constructed by adding the χ^2 values of contrast 1 and contrast 3 in Table 2, model A ($\chi^2 = 1.9323$, df = 2, $P < 0.100$). According to model A, none of the inbreeding classes had significantly different survival times.

Differences in the survival of individuals with different numbers of heterozygous loci were also tested. When survival functions associated with number of heterozygous loci were examined in the LIFETEST procedure (Table 2, model C), they were not significantly different ($P = 0.103$). There was, however, a 10.66-h difference in predicted MTTD between fish with zero and three heterozygous loci (Table 4). When both number of heterozygous loci and family were used as class variables in the LIFEREG procedure, number of heterozygous loci significantly affected survival time ($P = 0.0324$). This difference in significance between models B and C was due to the greater predictive power of the model when both family and number of heterozygous loci were included as class variables. In fact, model B was a significantly better fit of the data than a model with family as the only independent variable ($\chi^2 = 8.68$, df = 3, $P = 0.0391$).

Two single loci, *mIDHP-A** and *GPI-B**, significantly affected the predicted MTTD of an individual fish (Table 2, models D and E). The predicted MTTD for *GPI-B**66 homozygote fish was 13.51 h less than the predicted MTTD for

Table 4. Survival analyses for the effects of number of heterozygous loci per individual and for effects of *GPI-B** and *mIDHP-A** single locus genotypes.

	Label	df	χ^2	$P > \chi^2$	MTTD
Heterozygosity class	Overall	3	8.3835	0.0387	
	0	1	2.5059	0.1134	47.29
	1	1	0.2392	0.6248	39.28
	2	1	0.5326	0.4655	40.82
	3	0			36.63
*GPI-B**	Overall	2	8.4865	0.0144	
	66/66	1	4.7801	0.0288	28.00
	100/66	1	3.8292	0.0504	30.81
	100/100	0			41.51
*mIDHP-A**	Overall	4	11.8115	0.0188	
	100/100	1	0.8073	0.3689	37.09
	116/100	1	0.0971	0.7553	36.00
	116/134	1	0.2453	0.6204	36.00
	134/134	1	7.1178	0.0074	46.45
	134/100	0			39.26

Note: Each variable was analyzed separately. Because multiple loci were analyzed, a Bonferroni correction was used to correct for the probability of committing a type I error owing to multiple analyses.

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*GPI-B**100 homozyogtes (Table 4). The *mIDHP-A**134 homozygotes had a significantly later predicted MTTD than other *mIDHP-A** genotypes by as much as 10.45 h (Table 4).

Family effects could not be accounted for by single locus effects, particularly at the *mIDHP-A** and *GPI-B** loci. To test this, we first included the *mIDHP-A** and *GPI-B** loci as the only variables in a LIFEREG model. The *P* value associated with this model was 0.0018 as both loci had a significant effect on survival. When family was added as a variable in the model the *P* value associated with the locus effect was 0.657 and the *P* value associated with the family effect was 0.0001. Moreover, the fit of the model significantly improved when family was added ($\chi^2 = 137.78$, df = 35, $P < 0.005$). We also looked at the correlation between survival of a particular family given that family, and survival of a particular family given the *mIDHP-A** and *GPI-B** genotypes of individuals within that family. The slope of the line generated by this correlation was significantly different from 0, but the R^2 value was only 0.16. Both of these analyses suggest that family effects are not due to *mIDHP-A** and *GPI-B** locus effects.

Family effects were also not significantly confounded by the number of heterozygous loci. The slope of the line was not significantly different from 0 ($P = 0.275$) for the correlation between survival of family given the family grouping and survival of family given the number of heterozygous loci per individual within a family. The R^2 value for this correlation was 0.035.

Discussion

Family significantly affected fish survival in acute salinity stress in all of the models that tested for family effects (Table 2). One reason that family effects were observed in this salinity experiment is that the environmental variation illuminated familial differences in fitness-related traits. Differences between lineages cause them to respond to selection pressures, such as stress, differently so that familial differences in fitness-related traits are apparent in stressful environments but not necessarily in benign environments (Cohan and Hoffmann 1986).

Regardless of the inbreeding coefficient, family had a highly significant effect on survival time in salinity stress. The MTTD for particular families ranged from a low of 24.00 h in the $f = 0.125$ inbreeding group to a high of 96.62 h in the $f = 0.25$ inbreeding group. In fact, one effect of inbreeding is to increase variance among families within a particular inbreeding group (Falconer 1981). Inbreeding reapportions the variance so that as families become more inbred, genetic variance between families increases and genetic variance within families decreases (Falconer 1981; Hartl and Clark 1989). Some families will have greater fitnesses than average, but some will have much worse fitnesses.

In our experiment there was a positive relationship, although nonsignificant, between inbreeding coefficient and increasing variance in MTTD. A significant relationship between increase in variance and inbreeding coefficient is difficult to demonstrate because the experimental design requires a large number of lineages or families (Falconer 1981). Our experimental design would have had to include roughly three times as many families to show such a significant relationship. The observed positive relationship between inbreeding coefficient and variance was due to the much longer than average survival of some families in the inbred groups (Fig. 3). We also expected that the inbred groups would have included families with much shorter than average survival and that, on average, the TTD of inbred groups would have been shorter than the TTD of the outbred group. Families in the inbred groups did not have shorter TTDs as inbreeding coefficient increased, however. It appeared that up until 20 h all mosquitofish were able to compensate for the salinity stress. Therefore, in the inbred groups, the increase in variance among families was due to increased survival times but not to decreased survival times.

Inbreeding class did not significantly affect survival (Table 2, model A). Furthermore, inbred fish survived salinity stress longer than fish that were not inbred. This result sharply contrasts with previous studies of inbreeding (Falconer 1981; Ralls and Ballou 1983; Leberg 1990). Our results showed that the largest difference in the response, TTD, was between the $f = 0$ and $f = 0.25$ mosquitofish; however, it was only marginally significant. There are several possible reasons for this unexpected relationship between inbreeding coefficient and survival in salinity stress.

First, family effects swamped inbreeding effects. Family effects contributed to the positive relationship between inbreeding coefficient and predicted survival time. For example, if the subset of families with predicted survival times greater than 50 h is examined, two families in $f = 0.25$ and three families in $f = 0.125$ had MTTDs greater than 50 h. Only one family in $f = 0$ had an MTTD greater than 50 h. Family 90 from $f = 0.25$ had the greatest MTTD of 96.62 h, although the deletion of these data from analyses does not change the strong family effect. Clearly, large predicted survival times of several families within the inbreeding classes affected the overall predicted survival time of the inbreeding class itself, creating the unexpected relationship between inbreeding coefficient and survival in salinity stress.

Maternal effects may also account for differences in survival between outbred and inbred individuals. Maternal effects could be magnified by the different lengths of time that the inbreeding groups were raised in the laboratory. Females of inbred lineages that had been raised under optimal conditions in the laboratory for three generations could have had offspring that were more tolerant to stress than offspring of females that came from the field. Many studies exist that show a strong relationship between mother and offspring survival (e.g., Falconer 1981; Rossiter 1991; Glazier 1992). It is unlikely that the length of time that the mother was held in the laboratory could account for the positive relationship between inbreeding coefficient and survival, however. The MTTDs are larger in the $f = 0.25$ group than in the $f = 0.125$ group, although both groups of fish consist of lineages that were raised for the same length of time in the laboratory. It is likely that maternal effects are incorporated into family effects. Maternal effects are a common source of differences between families (Falconer 1981).

A significant lack of inbreeding effects may also have been due to pre-experiment selection through higher mortality of more inbred fish (homozygotes for deleterious alleles). In a related experiment, pre-experiment mortality of juveniles was not significantly different in the three inbreeding classes. Moreover, brood sizes were not significantly different for the

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three inbreeding classes, demonstrating that a disproportionately high number of embryos did not die prior to birth.

Finally, a significant lack of inbreeding effects may have arisen because the inbred and outbred fish stock originated from different populations and may have had a different genetic composition. Although the two populations had different allele frequencies, genetic distance in mosquitofish populations appears to be independent of geographic distance (McClenaghan et al. 1985). Furthermore, genetic diversity cannot be explained by habitat differences (McClenaghan et al. 1985). It is unlikely, then, that the site of origination of these populations significantly affected the results of this experiment.

Genetic variation may also affect survival of an individual in a particular environment. In our study, the number of heterozygous loci per individual was significantly related to survival in model B when family was included as a class variable. There was a general trend of increasing MTTD with decreasing number of heterozygous loci. The most homozygous class had a MTTD of 10.66 h later than that of the most heterozygous class. This is not the expected relationship. Two caveats should be included here, however. First, because the number of heterozygous loci is only significant when family is included in the model, number of heterozygous loci was not important by itself as a predictor of survival in salinity stress in mosquitofish. Second, the significance of number of heterozygous loci to survival in our study may largely have been due to the importance of several single-locus genotypes to survival under stress (Newman et al. 1989).

Genotypes of two of seven polymorphic loci (*mIDHP-A** and *GPI-B**) in this study significantly affected stress tolerance (Table 2). The 134/134 homozygote of *mIDHP-A** had a significantly greater MTTD than the other genotypes (Table 4). There was not an additive effect of the 134 allele, however, because the 134 allele in conjunction with other alleles was not significantly better than any of the other heterozygotes. Individuals heterozygous for the 116 allele had the poorest survival. Unfortunately, 116 alleles did not appear in the homozygous state in any of the individuals sampled. The *mIDHP-A** genotypes have been correlated with stress resistance in other studies of mosquitofish, though the effects appeared to be transient (Lee et al. 1992).

Rare homozygotes of the *GPI-B** locus (66/66 genotype) survived most poorly in the salinity stress, whereas common homozygotes (100/100) survived the best. This result is consistent with other studies on mosquitofish in stressful environments (Newman et al. 1989; Diamond et al. 1991; Heagler et al. 1993; Hughes et al. 1992). A relationship between survival in a particular stress and genotypes at the *GPI-B** locus exists in other organisms as well (Watt et al. 1983; Patarnello and Battaglia 1992). It may be that the other significant loci confer stress tolerance under particular stressors, while the *GPI-B** locus or linked loci are related to general stress tolerance (Newman et al. 1989; Hughes et al. 1991).

It is clear that the more information we have about a particular organism's genetics and breeding history, the better we can predict its survival under certain environmental conditions. The value of inbreeding coefficients to predict fitness of an organism has been well demonstrated (Ralls and Ballou 1983; Jarne and Delay 1990; Leberg 1990). Knowledge of genotypes at particular loci may be used to predict survival as well (Newman et al. 1989; Patarnello and Battaglia 1992). Our study, however, demonstrates that family significantly affected survival times and may outweigh any effects of inbreeding and genotype as a consistent predictor of survival under stressful environmental conditions. Inbreeding coefficient did not significantly predict survival, and the importance of number of heterozygous loci depended on whether family effects were incorporated into the model. Knowledge of family significantly increased the ability to predict survival of an individual. This point may have been overlooked in prior research on inbreeding because of a difficulty in assessing an individual's family.

Maintenance of genetic variation in populations, particularly by maintaining high levels of multilocus heterozygosity or by decreasing inbreeding, has been a central strategy of conservation management (Hedrick and Miller 1992). In part, this strategy seeks to preserve sufficient variation because (*i*) multilocus heterozygosity has been correlated with fitness-related traits and (*ii*) one of the causes of inbreeding depression is loss of variation owing to inbreeding. The evidence for a relationship between heterozygosity and fitness is circumstantial, however. Inbreeding depression, while severe in small captive populations, is not yet a major force in the demise of most natural populations. This study addresses the point that families or lineages may have greater effects on survival than either the number of heterozygous loci or inbreeding. It demonstrates that some families survive better in stressful environments than others, regardless of the inbreeding coefficient. These results do not mean that genetic diversity is unimportant to a population's viability. In fact, they suggest that much important variation is partitioned among families. Conservationists should maximize the numbers of lineages used in established captive and introduced populations.

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Do permit prices reflect the discounted value of fishing? Evidence from Alaska's commercial salmon fisheries

Daniel D. Huppert, Gregory M. Ellis, and Benjamin Noble

Abstract: We extend the analysis of Karpoff (J.M. Karpoff. 1984. Can. J. Fish. Aquat. Sci. 41: 1160–1166), who showed that Alaska salmon fishing permit prices during 1975–1981 were related to expected fishing returns in a fashion consistent with asset pricing theory. We develop a similar permit pricing model using permit prices and economic returns data for eight purse seine and gill-net fisheries over the period 1977–1990. A survey of fishing costs and activities from 1979 provides the data to estimate a cross-sectional relationship between fishing costs, vessel characteristics, and fishing practices. The cross-sectional cost equation is combined with fishery-specific vessel characteristics and operations during 1977–1990 to forecast costs over time. Finally, calculating net return as revenue minus cost, we estimate an adaptive expectations model relating net returns to permit prices. On the basis of tests of pooling across the two gear types, we conclude that the purse seine and drift gill-net fisheries should be modeled separately. The resulting estimates imply real annual rates of return of 11 and 6% for the drift net and purse seine fisheries, respectively. We view this as further evidence that license limitation can preserve economic rents in commercial fishing.

Résumé : Nous prolongeons l'analyse de Karpoff (J.M. Karpoff. 1984. J. can. sci. halieut. aquat. 41: 1160–1166) qui montrait que les prix des permis de pêche du saumon d'Alaska durant la période 1975–1981 étaient liés au rendement de la pêche conformément à la théorie des prix des éléments d'actif. Nous avons élaboré un modèle semblable des prix des permis en utilisant les données sur les prix des permis et le rendement économique pour huit pêches à la senne coulissante et au filet maillant au cours de la période 1977–1990. Un examen des coûts et des activités de pêche pour 1979 nous a fourni des données permettant d'évaluer la relation transversale entre les coûts de la pêche, les caractéristiques des bateaux et les méthodes de pêche. On combine l'équation du coût transversal aux opérations et aux caractéristiques des bateaux spécifiques à la pêche durant la période 1977–1990, en vue de prévoir les coûts à divers moments. Enfin, en calculant le rendement net en termes de la différence entre le revenu et le coût, nous estimons un modèle d'anticipations adaptatives liant le rendement net aux prix des permis. En se fondant sur des essais de regroupement des deux types d'engins, nous sommes arrivés à la conclusion que les pêches à la senne coulissante et au filet maillant dérivant devraient être modélisées séparément. Les estimations ainsi obtenues laissent supposer que le taux de rendement annuel réel est de 11 et de 6% pour la pêche au filet dérivant et à la senne coulissante, respectivement. À notre avis, ces résultats viennent confirmer que la limitation imposée par les permis permet de préserver les rentes économiques dans le domaine de la pêche commerciale.
[Traduit par la Rédaction]

Introduction

Alaska's commercial salmon fisheries are regulated under a system of license limitation (Schelle and Muse 1986). Tradeable permits were issued in 1974 for specific salmon fisheries corresponding to gear type and fishing area (e.g., Prince William Sound drift gill net or Southeast Alaska purse seine). Permit holders must be present on the vessel when fish are landed, and they must be "natural persons," not corporations. The state allows permit holders to own more than one type of permit, but no one may own more than one permit for any given fishery. If this license system prevents the dissipation of economic rents in the salmon fisheries, the theory of competitive markets for asset prices predicts that permit prices will reflect the discounted value of current and expected future net earnings generated by permit ownership and use. The earnings expectations are based upon anticipated economic and other conditions in the fishery (e.g., expected run sizes, expected market prices for fish, expected compensation for area closures). Testing this present value model of permit prices is important because, if it is found to be consistent with the available data, we can use permit prices to assess the success of license limitation in preserving economic rents.

There are two difficulties in testing the present value model of permit prices. First is the dearth of reliable cost and, hence, net earnings data. We overcome this obstacle by using cross-section survey data to estimate relationships between costs, vessel characteristics, and fishing practices. Then we construct a time series of operating and capital costs for each salmon fishery studied by forecasting costs from a time series on fishery-specific vessel characteristics and fishing practices. We combine the cost forecasts with annual gross revenues to construct a profitability (or net earnings) series for each fishery.

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D.D. Huppert.[1] School of Marine Affairs, University of Washington, Seattle, WA 98195-6715, U.S.A.
G.M. Ellis and B. Noble. Department of Economics, University of Washington, Seattle, WA 98195-3330, U.S.A.

[1] Author to whom all correspondence should be addressed.
e-mail: huppert@u.washington.edu

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Second, in the absence of a specific link between expected profits and existing information, the theory that current prices equal present value of expected future profits has few empirically testable implications. To create a testable theory we embed an adaptive model of profit expectations into the present value model of competitive permit pricing. Specifically, we assume that expected future profits are a weighted sum of past profits, and that the weights decline exponentially as profit information ages. Hence, our empirical work jointly tests whether the data are consistent with the present value model of permit pricing and with the adaptive model of expectations formation. We conclude that the data are consistent with the combined present value – adaptive expectations model of permit prices based upon the reasonable magnitudes of the estimated discount rates and expectations adjustment processes.

In a previous study of Alaska's commercial salmon fisheries Karpoff (1984) also estimated a present value model of permit prices that generated a discount for investments in fishing permits. Our study of the limited entry permit market represents an extension of and departure from Karpoff's earlier work in a number of important respects. First, our data cover more than twice as many years, including all of the 1980s, an important period for the rise in real permit prices. Second, our constructed cost series, while perhaps not ideal data, arguably represents an improvement relative to cost series constructed by Karpoff from the tenuous link between revenues and costs. Third, in his asset pricing model, Karpoff used nominal permit price and income data. This implicitly assumes that permit holders use a constant nominal rate of return to evaluate permit purchase decisions over time, even though his data covered the inflationary period from 1976 to 1981. In contrast, we use the gross national product price deflator (Council of Economic Advisors 1994) to deflate all dollar-denominated times series to 1979-equivalent dollars, so that our estimated rate of return is defined in real, not nominal, terms. Finally, when estimating his adaptive expectations model of profitability and permit price levels, Karpoff pooled panel data for purse seine and drift gill-net fisheries, thereby estimating the same model for both types of salmon fisheries. We test and then reject this pooling restriction in favor of an alternative hypothesis that permit prices are based on expectations of future profitability that are formed in different ways for purse seine versus gill-net permit holders.

This paper examines salmon fishing permit prices in Alaska during 1977–1990 and interprets the relationship between permit prices and expected net earnings in present value model asset pricing. Our objectives are (*i*) to determine whether over a longer span of time than previously examined permit prices continue to conform to the theory of asset pricing, (*ii*) to examine differential properties of longer term predictive models for two main classes of salmon fishing gear, and (*iii*) to carefully test empirical models of adaptive expectations of profitability and asset pricing for the presence of serial correlation. Given that our final models rely on a lagged dependent variable (permit price) for explanatory power, the potential for biased coefficient estimates of the models' parameters, owing to the presence of serial correlation, is a source for concern. We begin the paper with a presentation of our new data. Following that we focus on key features of our model that differ from Karpoff's, present our formal statistical models, and comment on the remaining sources of unexplained price variation. The results generally confirm that Alaska's permit price trends are consistent with simple asset pricing theories. The point estimate of the real rate of return that we calculate for the gill-net fisheries studied is approximately 11% and, as discussed below, this estimate is quite precise. For purse seine permits, the results are not as good. In particular, the rapid rise in prices during 1989 and 1990 is not well predicted by our model. When the data are restricted to the subsample years of 1977–1988, the estimated real rate of return to purse seine permits is approximately 6%, but this point estimate is relatively imprecise.

Data and methods

To construct the permit price model we use three sources of data, each derived from information collected and maintained by various Alaska state agencies. The data are used to generate time series for both permit prices and fishing profits (ex-vessel sales minus fishing costs) for 1977–1990. In each case we select for analysis the annual average data for the eight commercial salmon fisheries of interest: purse seine fishing in Southeast Alaska, Prince William Sound, Cook Inlet, and Kodiak; and drift gill-net fishing in Southeast Alaska, Prince William Sound, Cook Inlet, and Bristol Bay. The three data sources are as follows.

(*i*) The time series data on permit sales prices are based upon permit transfer records maintained by the state's Commercial Fishery Entry Commission. After deleting prices deemed to be nonmonetary (mainly gifts and inheritances), the Commission periodically releases the average price paid for permits (withholding information for fisheries having fewer than four transfers in the period). We use the annual average price published by the Commission's annual reports (Commercial Fishery Entry Commission 1992).

(*ii*) The data on average annual ex-vessel sales revenue per permit holder, average number of weeks fished in each fishery, and physical characteristics of fishing vessels used by permit holders in each year from 1977 to 1990 were collected by Commission staff from records of the Alaska Department of Fish and Game (ADFG).The average number of ex-vessel salmon sales per permit is derived from the state's fish ticket system, which tracks volume of commercial fish landings throughout the state and is the basis for levying the state's raw fish tax (Commercial Fishery Entry Commission, Basic Information Table 1A, January 17, 1992, printing). Vessel characteristics are derived from the state's vessel registration files (Commercial Fishery Entry Commission 1982, and in a report specially generated for us by Commission staff).

(*iii*) In 1979 the Alaska Sea Grant Program funded a survey of hand troll, power troll, drift gill-net, purse seine, and set gill-net fishermen in an effort to gain further information about fishermen's income. These surveys collected information regarding the physical characteristics of the vessels, fishing gear, and fishing effort (see Table 1). In addition, the surveys included questions pertaining to operating costs, earnings, and capital investments. The surveys were mailed to fishermen in five different areas: Southeast Alaska, Cook Inlet, Prince William Sound, Kodiak, and Bristol Bay. Of the 13 400 surveys distributed, approximately 14% were completed and returned. Throughout this analysis, it is assumed that these respondents represent a random sampling of eligible fishermen. A complete description of the survey instrument and a summary of results can be found in Larson (1980). These cost data, also used by Karpoff (1984), were provided to us directly by University of Alaska's Sea Grant Director.

Briefly, our method for preparing the time series data was as follows. The cost data described in (*iii*) were used to develop two linear, cross-sectional relationships between costs, fishing activity, and vessel characteristics. One linear function relates operating costs to vessel characteristics and fishing effort, and the other relates capital value of vessel and gear to vessel characteristics and fishery. These

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Table 1. Average annual fishing revenue, activity, costs, vessel values, vessel characteristics, and operational characteristics for eight Alaska salmon fisheries in 1979.

	Purse seine vessels	Gill-net vessels
Operational variables		
Gross revenue (1979 US$)	71 796	45 073
Weeks fished	11.6	12.7
Operating costs (1979 US$)	41 181	18 615
Capital value of vessel and gear (1979 US$)	158 048	70 626
Vessel characteristics		
Vessel age in years	17.6	9.4
Vessel size (gross t)	16.06	8.98
Vessel length (m)	11.9	9.8
Horsepower of main engine	214	237
Diesel engine (%)	91	54
Aluminum hull (%)	—	9.5
Fiber-glass hull (%)	44	59
Number of vessels in cost study		
Bristol Bay	—	252
Cook Inlet	14	122
Kodiak	52	—
Prince William Sound	27	24
Southeast Alaska	44	75

Note: Data from Larson (1980).

Table 2. Cross-section regression equations for annual operating costs of Alaska salmon vessels.

	Purse seine vessels		Gill-net vessels	
	Coefficient	*t* statistic	Coefficient	*t* statistic
Intercept	5 569	0.22	–4 240	0.47
Age of vessel	–32.1	0.14	4.05	0.03
Gross weight (t)	925.5	2.23	–271.2	1.16
Weeks fished	1 014	2.30	213	1.62
Length (m)	2 548.6	1.11	748	0.73
Horsepower	–33.2	1.46	12.7	1.27
Diesel[a]	6 684	0.51	–531	0.24
Aluminum[a]	—	—	–1 603	0.47
Fiber glass[a]	7 107	1.00	3 283	1.22
Bristol Bay[b]	—	—	26 418	8.37
Kodiak[b]	–33 149	5.33	—	—
Cook Inlet[b]	–16 882	1.65	–685.9	0.21
Southeast Alaska[b]	–34 611	3.45	2 292	0.65
R^2	0.483		0.474	
Sample size	70		263	

[a] Diesel engine dummy variable: excluded class is gasoline engine. Vessel hull material dummy variables: excluded class is wood or steel.

[b] Location dummy variables equal 1 for vessels fishing in the named area, zero otherwise. Excluded class is Prince William Sound.

linear functions were then used in conjunction with the time series on fishing vessel characteristics and fishing activity in (*ii*) to calculate the operating cost and capital investment per permit in years other than 1979. Subtracting the resulting "synthetic" operating cost time series from inflation-corrected gross revenue per permit creates the estimated net revenue per permit for 1977–1990 for the eight salmon fisheries. We elaborate on this method below.

Operating costs

The survey definition of operating costs includes all out of pocket expenses, including expenditures on fuel, crew wages or shares, insurance, and moorage. The opportunity wages of the owner–operator are not included in operating costs. Operating costs vary by type and size of vessel (e.g., hull type, engine size, fuel type) and with amount of fishing activity. More specifically, we assume that the operating costs for the years are a linear function of gear type, vessel characteristics, and fishing activity. The fishing activity indicators collected in the survey include the number of days committed to the fishery and the number of weeks spent in the fishery. These measures of fishing effort include not only the time spent fishing, but also the time spent in transit, in readying the vessel, and in repairing gear.

The linear operating cost equations include as independent variables the vessel characteristics, weeks fished, and dummy variables for location. Dummy variables representing fishery location are included to capture regional differences in the prices of fishing inputs and other unmeasured differences in fishing conditions. Linear regression equations for operating costs were estimated separately for the purse seine and gill-net fisheries (Table 2). The linear functional form was chosen because it facilitates using the equation to calculate a cost time series based upon the annual ADFG data for 1977–1990. Average vessel characteristics for each year are simply substituted into the linear cost equation. The linear specification guarantees that the calculated average cost is invariant to whether the average is computed by inserting annual fleet wide average data into the cost equation or by averaging linear cost calculations across individual vessel data. Assuming that the cost equation estimated from 1979 is stable across time, these results provide time series estimates of average operating costs and average capital investment costs.

The explanatory power of these models is fairly high. Approximately 50% of the variation in operating costs is explained by this simple linear specification. The results for the purse seine fishery confirm that both boat attributes and fishing effort have individually significant impacts on operating costs. Increased gross tonnage has a significant positive impact on operating costs, as does an increased time commitment to the fishery. The gill-net analysis confirms our expectations that operating costs vary by location, but does not demonstrate the individual significance of boat characteristic or fishing effort variables. However, overall explanatory power is still strong.

Capital value

The reported capital values of fishing vessels and gear reflect the fishermen's assessment of the market value in 1979 and do not include the value of the fishing permit. Again, we estimate a linear equation relating capital value of vessel and gear as reported in 1979 to vessel characteristics. Fishing effort is not included, because effort does not directly affect the value of the fishing equipment. In addition, location variables are not included because the ease with which fishing capital can be moved from one location to another suggests that the relationship between capital value and vessel characteristics should not vary by location. As with the operating cost equation, the simple linear form was chosen because we need to use it in conjunction with average annual vessel characteristic data to generate a capital cost series for the years 1977–1990. The cross-sectional linear regressions for capital costs (Table 3) confirm that boat characteristic data can explain a large portion of the variation in capital values. In general, these characteristics are individually significant and have effects that match intuitive expectations. For example, older vessels are worth less, while increased boat size and engine power increase capital value.

Forecasting operating costs, capital values, and net revenues

As noted above, the time series of fishing costs and vessel values are created by simple substitution of the vessel characteristics and fishing

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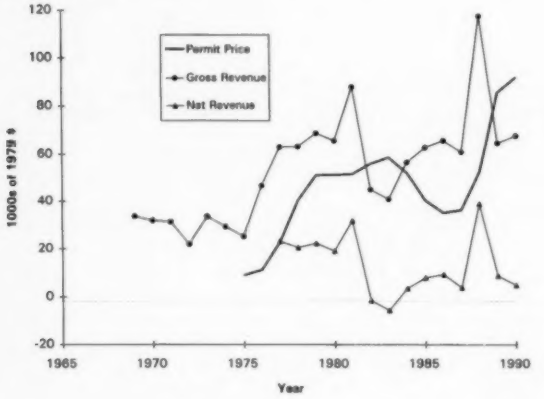
Table 3. Cross-section regression equations for capital value of fishing vessels and gear.

	Purse seine vessels		Gill-net vessels	
	Coefficient	*t* statistic	Coefficient	*t* statistic
Intercept	–106 100	1.63	109 030	5.40
Age	–1 610	2.22	–1 002	3.06
Vessel size (t)	7 873	5.74	2 668	4.89
Vessel length (m)	12 999	2.04	12 713	5.55
Main engine horsepower	38.0	0.52	79.7	3.66
Diesel	–22 363	0.65	21 429	4.39
Fiber glass	52 952	2.34	13 393	2.64
Aluminum			20 273	2.31
R^2	0.706		0.625	
Sample size	70		263	

Fig. 1. Purse seine permits: weighted average price, gross revenue, and net revenue per permit for four Alaska salmon fisheries. (Each fishery given weight in proportion to number of purse seine permits.)

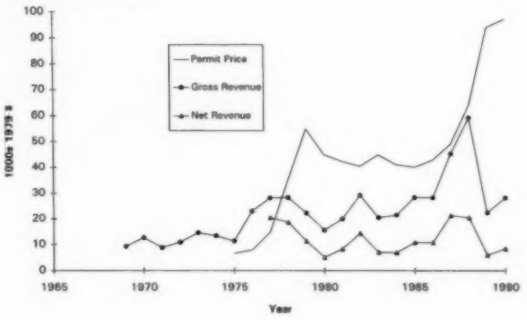


activity time series data into the estimated linear equations. For consistency, it is important that vessel characteristics and effort in the 1979 survey be equivalent to corresponding time series data derived from the ADFG landings records and vessel registration files. On the basis of the data definition and collection methods, the information on vessel characteristics from the two sources is essentially identical. However, the fishing activity information from the two sources is not the same. The time series data on weeks of fishing reflect the number of weeks in which permit holders recorded fish landings, while the survey data on weeks fished more broadly cover time committed to the fishery "including readying the vessel, repairing gear, standing by for opening, etc." To reconcile the differences between the two measures of fishing activity, we calculated the ratio of reported weeks committed to fishing from the 1979 survey to average weeks fished from the ADFG data for 1979. Assuming that this ratio would remain constant over 1977–1990, we scaled the annual data on weeks fished to reflect the total time commitment to fishing.

A model of permit prices

In this section of the paper, we present our model of permit prices combining the present value of expected profit and adaptive expectations models. As shown in Figs. 1 and 2, the model needs to explain a pattern of permit prices for purse seine and drift gill-net permits that exhibits a rapid rise during 1975–1979, then a period with no strong trend, followed by an extremely rapid rise after 1987. Except for a slight difference in notation to reflect our computation of the operating and capital cost series, our model has the same basis as that of Karpoff (1984). If permits are treated as capital investments, then the price a risk-neutral fisherman is willing to pay for a permit is the present value of the profit stream expected to be generated by exercising the right to fish under that permit. Let P_t be the permit price in period t, R_t the gross revenue from fishing in period t, C_t the operating cost for period t, and K_t the capital value of the boat and other equipment used by the fisherman in period t. Then the following equation describes the willingness to pay for a permit:

Fig. 2. Drift gill-net permits: weighted average price, gross revenue, and net revenue per permit for four Alaska salmon fisheries. (Each fishery given weight in proportion to number of gill-net permits.)



$$(1) \qquad P_t = \sum_{i=0}^{\infty} \frac{E_t(R_{t+i} - C_{t+i} - \gamma K_{t+i})}{(1+r)^i}$$

where E_t is the expectation operator for period t, r is the annual rate of discount, and γ is the parameter that converts capital value into the annual capital costs attributable to the fishery in question. The parameter γ is determined by rates of discount and depreciation and the fact that many fishermen will work more than one permit and fishery in a given year with the same stock of capital. The numerator in the summation expression is the level of profit expected to prevail in period $t + i$ given the information available in period t. When the most reasonable expectation is that current and future annual profit levels will remain constant (i.e., there is no a priori reason to expect future profit levels to systematically rise or fall), then eq. 1 may be simplified as a perpetuity:

$$(2) \qquad P_t = \frac{E_t[\pi]}{r}$$

where $E_t[\pi] = E_t[R_t - C_t - \gamma K_t] = E_t[R_{t+i} - C_{t+i} - \gamma K_{t+i}]$ for all $i \in \{1, 2, 3, \ldots\}$. Equation 2 states that permit prices are a function of expectations of long-run profitability. Profits in future periods will no doubt vary; however, eq. 2 is merely a reflection of the hypothesis that a current expectation of steady future profits is as reasonable as any other systematic expectation.

Following Karpoff (1984), we develop an adaptive expectations model for the formulation of $E_t[\pi]$, and then we employ a Koyck transformation to express the model in estimable form. In an adaptive expectations framework, it is hypothesized that fishermen will revise their previous period's expectation of annual profitability by some proportion of its deviation from the ex-post realization of the period's profit. That is,

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$$(3)\quad E_t[\pi] - E_{t-1}[\pi] = \beta(\pi_{t-1} - E_{t-1}[\pi]) \text{ or } E_t[\pi] = \beta\pi_{t-1} + (1-\beta)E_{t-1}[\pi]$$

where $\beta \in \{0, 1\}$ is the parameter of expectations adjustment. After lagging eq. 3 and then using a process of backwards recursion, eq. 3 may be rewritten as

$$(4)\quad E_t[\pi] = \beta\pi_{t-1} + \beta(1-\beta)\pi_{t-2} + \beta(1-\beta)^2\pi_{t-3} + \ldots = \beta\Sigma(1-\beta)^i\pi_{t-1-i}$$

where $\pi_{t-1-i} = (R_{t-1-i} - C_{t-1-i} - \gamma K_{t-1-i})$ for $i \in \{0, 1, 2, \ldots\}$. Following Karpoff's notation, we let $\lambda = 1 - \beta$, where λ may be interpreted as a measure of how heavily profit realizations from distant periods influence current expectations of future profitability. The higher the λ, the greater the weight placed on a long-run history of profitability. Conversely, a small λ is consistent with expectations of future profitability, $E_t[\pi]$, that are based heavily on the most recent profit realizations.

Equation 4 may be rearranged in a convenient form for statistical estimation through the use of a transformation developed by Koyck (1954). Using eq. 2, eq. 4 may be rewritten as

$$(5)\quad P_t = \frac{(1-\lambda)\sum_{i=0}^{\infty}\lambda^i\pi_{t-1-i}}{r}$$

or in terms of lagged permit price, we have

$$(6)\quad \lambda P_{t-1} = \frac{(1-\lambda)\sum_{i=0}^{\infty}\lambda^{i+1}\pi_{t-2-i}}{r}$$

Consequently, by subtracting eq. 6 from eq. 5, and then adding λP_{t-1} to both sides of the resulting expression, we obtain the estimable form of the asset pricing model that embodies the adaptive expectations hypothesis about future profitability:

$$(7)\quad P_t = \frac{(1-\lambda)}{r}\pi_{t-1} + \lambda P_{t-1}$$

The adaptive expectations hypothesis implies that the permit price in period t may be explained solely in terms of the realization of profit and permit price in period $t-1$. This is essentially because the permit price in period $t-1$ already embodies the expectations of future profitability that are due to the realizations of profit prior to period $t-1$. At the beginning of period t, the only new information that should influence P_t relative to its prior level, P_{t-1}, is the deviation of expected profit from its actual level in period $t-1$, namely $E_{t-1}[\pi] - \pi_{t-1}$. Finally, assuming the existence of additive error terms, eq. 7 may be estimated by ordinary least squares under a maintained hypothesis of an absence of serial correlation among those error terms.

As a practical matter, in estimating eq. 7, a couple of options are available concerning the treatment of the discount rate r. If, as a researcher, one were relatively certain of the expected rate of return demanded of investments in limited entry permits, one could exogenously impose that rate for r. Then estimating eq. 7 would consist of regressing permit price on lagged permit price and a variable that is equal to lagged profit divided by the exogenously imposed estimate of r. The resulting coefficients could be given the interpretation of estimates of λ and $\beta = 1 - \lambda$, respectively. In this paper, we, like Karpoff, have chosen a different route for the estimation of the model's parameters. Without reliable prior information on the value of the discount rate used by salmon fishermen in Alaska, we simply regress permit price on lagged permit price and lagged profit. Consequently, the coefficient estimates are of λ and $(1-\lambda)/r$, respectively. Using the estimate of λ, we then infer the rate of return r that rationalizes the hypothesized asset pricing model. The credibility of the pricing model then amounts to a test for the reasonableness of the inferred rate of return.

In estimating eq. 7, two different measures of lagged profitability may be utilized. The first measure is lagged profit as previously defined: $\pi_{t-1} = R_{t-1} - C_{t-1} - \gamma K_{t-1}$. In this formulation, lagged profit consists of lagged gross revenue less lagged operating and capital cost. The interpretation of the estimated discount rate r is that of the expected rate of return on an investment in a limited entry permit. Alternatively, one may use lagged net revenue as a measure of lagged profit (i.e., $\text{NET}_{t-1} = R_{t-1} - C_{t-1}$): the difference between lagged gross revenue and lagged operating cost. If this measure, which does not include the capital cost of using a boat and other equipment in exercising the permit in question, is used in place of π_{t-1}, then the estimated discount rate r must be interpreted as the expected rate of return necessary to cover both the opportunity cost of money invested in the permit and the opportunity cost of capital used in the associated fishery.

A test for pooling purse seine and drift gill-net data

When data for purse seine and drift gill-net salmon fisheries are first pooled and a single asset pricing equation is then estimated, the results, in terms of their economic content, are rather disappointing. Equations 8 and 9 are the results of ordinary least squares estimation of eq. 7, with lagged profit and lagged net revenue used as measures of π_{t-1} (t statistics are reported in parentheses):

$$(8)\quad P_t = \underset{(4.17)}{0.279}\text{NET}_{t-1} - \underset{(0.14)}{0.0019}K_{t-1} + \underset{(26.38)}{1.035}P_{t-1}$$

and

$$(9)\quad P_t = \underset{(4.43)}{0.281}\text{NET}_{t-1} + \underset{(35.72)}{1.038}P_{t-1}$$

With or without annual capital costs included in the asset pricing equation, the estimates of λ, the coefficient on lagged permit price from eq. 7, are greater than one. These results are difficult to interpret in any meaningful way. Recall that $\lambda = 1 - \beta$, and that $\beta \in \{0, 1\}$ is the parameter of expectations adjustment from the adaptive expectations model of profitability. Economic theory suggests that λ should be less than one; if one believes in the credibility of an estimate of λ that is greater than one, then one is forced to believe that fishermen use a negative real rate of discount when evaluating permit investment decisions. Alternatively, the model may be misspecified. In particular, estimating a single asset pricing equation for both purse seine and gill-net fisheries may be the source of the problem.

The hypothesis that the data for purse seine and drift gill-net salmon fisheries may be legitimately pooled, implying that a single adaptive expectations model of profitability is sufficient to explain permit prices in both types of fisheries, is tested in this section of the paper. Three interactive slope dummy variables are added to the asset pricing model of eq. 7: PSP, PSNET, and PSK. They are the product of a dummy variable indicating whether an observation is from a purse seine fishery (1 = yes and 0 = no) and the relevant observation of permit price (P), net revenue (NET), and capital value (K), respectively. One test for the legitimacy of pooling is simply whether any of the coefficient estimates for these interactive dummy variables is statistically different than zero. If different than zero, such coefficient estimates would suggest that a single estimated asset pricing equation, designed to explain both purse seine and gill-net permit pricing behavior, will possess biased parameter estimates. Alternatively, one can test the hypothesis that the coefficients on all of the interactive dummy variables are zero. The joint test statistic under this null hypothesis has an F distribution, and in all of the regressions used to test the legitimacy of pooling purse seine and drift gill-net data that follow, the null hypothesis is rejected at the 5% level of significance.

The first diagnostic equation estimated includes pooled data from all of the sample years 1977–1990:

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Table 4. Permit price model for drift gill-net fisheries.

	Coefficient	Standard error	*t* statistic
Capital value included in measure of lagged profit[a]			
Net earnings, NET_{t-1}	0.6796	0.1676	4.05
Capital value, K_{t-1}	−0.0007	0.0712	0.01
Lagged price, P_{t-1}	0.9274	0.0752	12.33
Capital value not included in measure of lagged profit[b]			
Net earnings, NET_{t-1}	0.6788	0.1454	4.67
Lagged price, P_{t-1}	0.9269	0.0457	20.29

Note: Current year permit price is dependent variable.

[a] R^2, 0.7897; sample size, 52; Breusch-Godfrey Lagrange multiplier test statistic, 1.22.

[b] R^2, 0.7897; sample size, 52; Breusch-Godfrey Lagrange multiplier test statistic, 1.20.

$$(10)\quad P_t = \underset{(3.76)}{0.680}\mathrm{NET}_{t-1} - \underset{(0.01)}{0.0007}K_{t-1} + \underset{(11.43)}{0.927}P_{t-1} - \underset{(2.29)}{0.450}\mathrm{PSNET}_{t-1} - \underset{(0.09)}{0.0073}\mathrm{PSK}_{t-1} + \underset{(1.62)}{0.172}\mathrm{PSP}_{t-1}$$

Again, *t* statistics for the coefficient estimates are in parentheses. In particular, the hypothesis that the coefficient on $PSNET_{t-1}$ is equal to zero is easily rejected at the 5% level of significance. The statistically significant coefficient on $PSNET_{t-1}$ suggests that purse seine fishermen give much less weight to the previous period's realization of net revenue when revising their expectation of long-run profitability than do gill-net fishermen. The implication is that the two types of fishermen form expectations in dramatically different ways.

The next asset pricing equation estimated excludes the data on capital costs, and once again, the hypothesis that purse seine and drift gill-net fishermen form expectations about future profitability (specifically, net revenues) in the same way is strongly rejected:

$$(11)\quad P_t = \underset{(4.32)}{0.679}\mathrm{NET}_{t-1} + \underset{(18.79)}{0.927}P_{t-1} - \underset{(2.71)}{0.463}\mathrm{PSNET}_{t-1} - \underset{(2.35)}{0.148}\mathrm{PSP}_{t-1}$$

The hypotheses that the coefficients on $PSNET_{t-1}$ and PSP_{t-1} are equal to zero are both easily rejected at the 5% level of significance.

Because permit prices rose so dramatically in the last 2 years of our sample period (1989 and 1990), perhaps pooling of gill nets and purse seines is justified only for the subset of data observed prior to the significant permit price increases. Consequently, we tested the pooling hypothesis in one more set of regressions. For these regressions, the underlying data sample is from the truncated period beginning in 1977 and ending in 1988:

$$(12)\quad P_t = \underset{(3.94)}{0.612}\mathrm{NET}_{t-1} - \underset{(0.03)}{0.0018}K_{t-1} + \underset{(10.72)}{0.917}P_{t-1} - \underset{(2.28)}{0.398}\mathrm{PSNET}_{t-1} + \underset{(0.11)}{0.0076}\mathrm{PSK}_{t-1} + \underset{(0.47)}{0.050}\mathrm{PSP}_{t-1}$$

and

$$(13)\quad P_t = \underset{(4.44)}{0.610}\mathrm{NET}_{t-1} + \underset{(19.05)}{0.915}P_{t-1} - \underset{(2.47)}{0.384}\mathrm{PSNET}_{t-1} + \underset{(1.17)}{0.071}\mathrm{PSP}_{t-1}$$

Again, with or without measures of capital value for boat and equipment included, the estimated coefficients on $PSNET_{t-1}$ are statistically different from zero. We reject the hypothesis that a single equation should be estimated to explain permit prices in both purse seine and gill-net fisheries.

In addition to the aforementioned tests for pooling of purse seine and drift gill-net data, we also introduced slope dummy variables for each geographic area to test the legitimacy of pooling the data across geographic boundaries. We cannot reject the hypothesis that the coefficients on the geographic slope dummies are all zero. That is, we cannot reject the hypothesis that fishermen from different areas form expectations of future profitability in similar ways. Consequently, in estimating separate models for purse seine and drift gill-net fisheries, we pool observations from Southeast Alaska, Prince William Sound, Cook Inlet, Kodiak, and Bristol Bay. In the approach that follows, we estimate two separate adaptive expectations – asset pricing models: one for the four purse seine fisheries and one for the four drift gill-net fisheries.

Results

Drift gill-net fisheries

When eq. 7 is estimated with only data for the drift gill-net fisheries, the results may be given a reasonable economic interpretation. Table 4 presents results for the pricing model with lagged capital value included in the measure of lagged profit. The coefficient estimate on lagged permit price is $\hat{\lambda} = 0.927$, while the coefficient estimate on lagged net revenue is $(1 - \hat{\lambda})/r = 0.680$. These results imply an expected real rate of return equal to 10.68%. This rate of return may also be obtained directly with a nonlinear least squares estimation of eq. 7. The standard error and *t* statistic for this estimate of *r* are 0.1138 and 0.94, respectively. When lagged capital costs are included in the measure of lagged profit, the standard errors on all estimated coefficients are quite large, and the estimated coefficient on capital value is statistically indistinguishable from zero.

In contrast, when permit price is regressed against only lagged net revenue and lagged permit price, the comparable coefficient estimates are almost unchanged, but they are nearly twice as precise. Results for this case, in which capital costs of boat and equipment are excluded from the measure of profit, are also reported in Table 4. The estimated real rate of return in this model is 10.78%, with a standard error of 5.36% and a corresponding *t* statistic of 2.01. The hypothesis that the real rate of discount employed by drift gill-net fishermen is zero is rejected at the 5% level of significance. For the drift gill-net fisheries, including the capital value variable does very little to explain the variance in permit prices, and it reduces the precision of the coefficients of the other variables. This lack of explanatory power may be attributable in part to the low variation in our constructed capital value series, which evolves slowly over time as vessel characteristics change.

Information pertaining to the lack or presence of serial correlation in the models' error structure was also examined. As a diagnostic tool, we checked correlograms of the models' regression residuals and noted their consistency with a hypothesis of white noise regarding the models' error terms. Table 4 contains a formal test for first-order serial correlation. The Breusch (1978) – Godfrey (1978) Lagrange multiplier (LM) test statistics for the pricing models with and without capital costs are 1.22 and 1.20, respectively, and they are distributed χ^2 values with one degree of freedom. With a critical value of 3.84 at the 5% level of significance, the null hypothesis of no serial correlation cannot be rejected in either case. Additional LM tests for second-, third-, and fourth-order serial correlation also lead to the same conclusion: the null hypothesis of an absence of serial correlation cannot be rejected. This

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is important given the presence of the lagged dependent variable (permit price) in eq. 7. If serial correlation were present, the estimate of λ could be severely biased as serial correlation would propagate the error term through the dependent variable over time. With a lack of significant serial correlation in the models' error structures, our ordinary least squares results suggest that an adaptive expectations model of profitability and simple asset pricing equation explain the data for the drift gill-net salmon fisheries quite well. Therefore, a standard generalized least squares (GLS) procedure for correcting bias due to serial correlation, in particular, first-order autocorrelation, results in very little modification to the model estimated by ordinary least squares. The equation estimated by GLS is $P_t = 0.688\text{NET}_{t-1} + 0.907P_{t-1}$ with $\hat{\sigma} = 0.182$, implying a real discount rate of 13.52%.

Purse seine fisheries

The adaptive expectations – asset pricing model does not explain the purse seine fisheries data nearly as well. For the entire sample period (1977–1990), we estimate the following permit price equations:

$$(14)\quad P_t = \underset{(2.84)}{0.230}\text{NET}_{t-1} - \underset{(0.41)}{0.0080}K_{t-1} + \underset{(14.79)}{1.010}P_{t-1}$$

and

$$(15)\quad P_t = \underset{(2.98)}{0.215}\text{NET}_{t-1} + \underset{(25.38)}{1.075}P_{t-1}$$

As previously discussed, the finding of $\hat{\lambda} > 1$ is problematic. The result is due, in large measure, to the rapid rise in real purse seine permit prices in 1989 and 1990 (Fig. 1). A variety of factors might be responsible for a discrete change in expectations (of the value of permits) that may have occurred in 1989. The Exxon-Valdez oil spill in February 1989 introduced uncertainty about area closures and the promise of compensation, and beginning in 1988, the market for salmon saw very large price increases, to name but a couple of possible explanations for the rapid rise in permit prices.

Why these or other factors would influence the price of purse seine permits to a greater extent than the price of drift gill-net permits is unclear, but when dummy variables for the years 1989 and 1990 are introduced for each purse seine fishery, the adaptive expectations – asset pricing model reasserts its explanatory power. These results are reported in Table 5. The dummy variable coefficients for Prince William Sound and Cook Inlet for 1989 and 1990 are particularly significant. These areas were most directly affected by the oil spill, and the potential for compensation may explain the rapid rise in their permit values. Suffice it to note that without the introduction of dummy variables for 1989 and 1990, the simple asset pricing model does not explain well the purse seine permit price data for those years.

The coefficient estimates on lagged net revenue and lagged permit price obtained in these regressions are identical to those obtained by restricting the sample to the years 1977–1988 (without the need for 1989–1990 dummy variables). The point estimates for the real rate of discount in these models, with and without capital values as regressors, are reasonable: 15.20 and 6.10%, respectively; however, the associated standard errors are very large: 31.27 and 16.24%. Consequently, hypothesis tests have little power. For example, the hypothesis that the relevant rates of discount are zero cannot be rejected.

Table 5. Permit price model for purse seine fisheries.

	Coefficient	Standard error	*t* statistic
Capital value included in measure of lagged profit[a]			
NET_{t-1}	0.2142	0.0819	2.62
K_{t-1}	0.0058	0.0156	0.37
P_{t-1}	0.9674	0.0637	15.20
Southeast Alaska, 1989	1 484	12 438	0.12
Southeast Alaska, 1990	–2 746	13 594	–0.20
Prince William Sound, 1989	59 321	12 403	4.78
Prince William Sound, 1990	23 664	13 774	1.72
Cook Inlet, 1989	6 272	12 334	0.51
Cook Inlet, 1990	58 485	12 227	4.78
Kodiak, 1989	17 733	15 190	1.17
Kodiak, 1990	7 210	12 627	0.57
Capital value not included in measure of lagged profit[b]			
NET_{t-1}	0.2262	0.0744	3.04
P_{t-1}	0.9862	0.0378	26.12
Southeast Alaska, 1989	2 510	11 983	0.21
Southeast Alaska, 1990	–2 650	13 436	–0.20
Prince William Sound, 1989	59 191	12 256	4.83
Prince William Sound, 1990	22 653	13 343	1.70
Cook Inlet, 1989	5 809	12 130	0.48
Cook Inlet, 1990	58 170	12 058	4.82
Kodiak, 1989	16 634	14 724	1.13
Kodiak, 1990	7 560	12 447	0.61

[a] R^2, 0.8780; sample size, 48; Breusch-Godfrey Lagrange multiplier test statistic, 2.27.

[b] R^2, 0.8776; sample size, 48; Breusch-Godfrey Lagrange multiplier test statistic, 1.88.

Formal LM tests for the presence of serial correlation are presented in Table 5, and as in the drift gill-net case, the hypothesis of an absence of serial correlation is not rejected. A GLS correction for first-order autocorrelation changes the OLS estimates very little. For instance, the point estimate for the implied real rate of discount in the model without capital costs is 7.67% under the GLS procedure. Ordinary least squares estimation of the adaptive expectations – asset pricing model for purse seines generates sensible (albeit imprecise) parameter estimates, but only when the data sample is essentially restricted to the years 1977–1988. As is true for the drift gill-net fisheries model, the inclusion of capital costs for boat and equipment adds substantial imprecision to the coefficient estimates for lagged net revenue and permit price. Moreover, the hypothesis that the coefficient on capital value is zero cannot be rejected.

Comparing results for drift gill-net and purse seine fisheries

To compare the asset pricing models for drift gill-net and purse

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seine fisheries, we restrict attention to the relatively precise models without capital costs included in the measure of lagged net revenue. The estimated coefficients on the lagged permit price variable are 0.927 for the drift gill-net fisheries and 0.986 for the purse seine fisheries. To compare parameters of the adaptive expectations process described by eq. 4, recall that $\beta = 1 - \lambda$, where β is the weight placed on last period's net revenue in forming the current period's expectation of long-run per period profitability. For drift gill-net fisheries we estimate $\beta = 0.073$, whereas for purse seine fisheries we estimate $\beta = 0.014$. Drift gill-net fishermen apparently place more weight on recent net revenues when forming expectations than do purse seine fishermen. Differences in variability of annual net revenues for purse seine and drift gill-net fisheries may explain this difference in the formation of expectations. Measured in constant 1979 U.S. dollars, the standard deviation of average annual net revenue for purse seine fishermen, over the period 1977–1990, is $29 365, versus only $11 729 for drift gill-net fishermen. The mean of average annual net revenue in the two types of fisheries, calculated over the same period, differs by only $2532 (i.e., $14 532 for drift gill net and $12 000 for purse seine). Consequently, purse seine fishing for salmon may appropriately be viewed as a riskier prospect than drift gill-net fishing, and the weight placed on a single year's profit realization when updating expectations of long-run profitability is, quite understandably, smaller for purse seine fishing.

Discussion

One purpose of license limitation is to reduce the incentive for fishing vessel owners to overinvest in fishing capacity. If successful at forestalling the overcapitalization, license limitation programs will preserve resource rents, and these rents will be capitalized into the value of permits. However, economic studies generally conclude that permit programs can be only partially successful at achieving this objective (Anderson 1985; Wilen 1988; Campbell and Lindner 1990). While the license program holds the number of firms below the level naturally occurring with open access, each firm continues to compete for a share of the overall harvest, often by increasing catch capacity, a process that increases both capital and operating costs per permit. The amount of rent creation attributable to license limitation will vary among fisheries depending on the degree to which this competition for shares results in increased costs. Resource rent is likely to be greatest in fisheries having relatively low gear flexibility, hence experiencing steeply rising marginal costs of fishing capacity expansion (Wilen 1988). Low gear flexibility would seem to describe the drift gill-net fishing vessels, especially where the length of net per vessel is restricted as in Prince William Sound. Since the prevalence of rents is indicated by adherance of permit prices to asset market theory, our test of the asset pricing model is also a test of whether the Alaska permit system facilitates the creation of permanent economic rent in the salmon fisheries. At least for the drift gill-net fisheries, we find convincing evidence that the permit system has generated permanent economic rent. The absolute magnitude of the rents and a comparison of actual to potential resource rents should be the focus of additional study.

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Occurrence and diet of killer whales in northern Norway: seasonal patterns relative to the distribution and abundance of Norwegian spring-spawning herring

Tiu Similä, Jens Christian Holst, and Ivar Christensen

Abstract: Our objectives were to investigate the seasonal occurrence of photoidentified killer whale pods in relation to the distribution of Norwegian spring-spawning herring and whether or not pod-specific differences in the occurrence or diet of killer whales could be demonstrated. In a 4-year study, the killer whales occurred in different areas during the summer and the fall–winter, and these areas coincided with the distribution areas of herring. Killer whales were encountered most frequently during October–January when the whole adult and part of the adolescent stock of spring-spawning herring wintered in the study area. Thirty-nine killer whale pods were identified, and generally the same pods were encountered each year of the study. Differences in the timing and areas of occurrence of pods could be demonstrated. Adult and adolescent herring seemed to be the main prey of the killer whales during both fall–winter and summer. Six new species were added to the list of known prey species of Norwegian killer whales. The present pattern of seasonal occurrence of killer whales in the coastal waters of northern Norway is expected to change as a result of growth in the Norwegian spring-spawning herring stock.

Résumé : Nous avions comme objectifs d'étudier l'apparition saisonnière de groupes d'épaulards photo-identifiés relativement à la distribution des harengs norvégiens se reproduisant au printemps, et de déterminer s'il y avait des différences spécifiques aux groupes dans le nombre ou le régime alimentaire des épaulards. Lors d'une étude de 4 ans, les épaulards ont été observés à différents endroits à l'été et pendant la période automne–hiver, et ces endroits correspondaient à l'aire de distribution des harengs. On observait surtout des épaulards durant la période allant d'octobre à janvier lorsque la totalité du stock d'adultes et qu'une partie du stock des adolescents de harengs se reproduisant au printemps passaient l'hiver dans l'aire d'étude. Trente-neuf groupes d'épaulards ont été dénombrés, et ce sont surtout les mêmes groupes qui étaient observés chaque année de l'étude. On a fait état de différences dans les moments et les endroits où les groupes étaient observés. Les harengs adultes et adolescents semblaient constituer la principale proie des épaulards, tant pendant la période automne–hiver qu'à l'été. Six nouvelles espèces ont été ajoutées à la liste des proies connues des épaulards norvégiens. La distribution actuelle des épaulards dans les eaux côtières du nord de la Norvège changera, prévoit-on, à la suite de la croissance du stock de harengs norvégiens se reproduisant au printemps.
[Traduit par la Rédaction]

Introduction

Killer whales (*Orcinus orca*) are found in all oceans and feed on a variety of prey including several kinds of fish, cephalopods, pinnipeds, cetaceans, and birds (Matkin and Leatherwood 1986). Although a generalist as a species, killer whale populations behave as specialists, following seasonal movements of their preferred prey (Condy et al. 1978; Berzin and Vladimirov 1983; Sigurjonsson et al. 1988; Felleman et al. 1991; Guinet 1991; Ford et al. 1994). However, no killer whale population has been studied throughout the year and little is known about possible seasonal changes in their diet. Diet preferences of killer whales may vary within the same geographical area; for example, separate populations with differences in their diet and distribution pattern have been found off British Columbia and Washington state (Bigg et al. 1990; Felleman et al. 1991; Ford et al. 1994), Alaska (Ellis 1987), and the Antarctic (Berzin and Vladimirov 1983).

In Northeast Atlantic waters, the occurrence of killer whales is associated with the presence of herring (*Clupea harengus*) in Iceland (Sigurjonsson et al. 1988), of herring and salmon (*Salmo* spp.) in waters around the British Isles (Evans 1988), of herring and mackerel (*Scomber scombrus*) around the Faroe Islands (Bloch and Lockyer 1988), and of herring along the coast of Norway (Jonsgård and Lyshoel 1970; Christensen 1982, 1988; Øien 1988). Other known types of prey in Norwegian waters are cod (*Gadus morhua*), squid, seals (Christensen 1978), and bottlenose whales (*Hyperoodon ampullatus*) (Jonsgård 1968).

Christensen (1988) estimated that a minimum of 1500 killer whales might be present in Norwegian coastal waters when herring is abundant. Seasonal peaks in the abundance of herring

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T. Similä.[1] Norwegian College of Fisheries Science, University of Tromsø, N-9037 Tromsø, Norway.
J.C. Holst and I. Christensen. Institute of Marine Research, P.O. Box 1870 Nordnes, N-5024 Bergen, Norway.

[1] Author to whom all correspondence should be addressed. e-mail: tiu@nfh.uit.no

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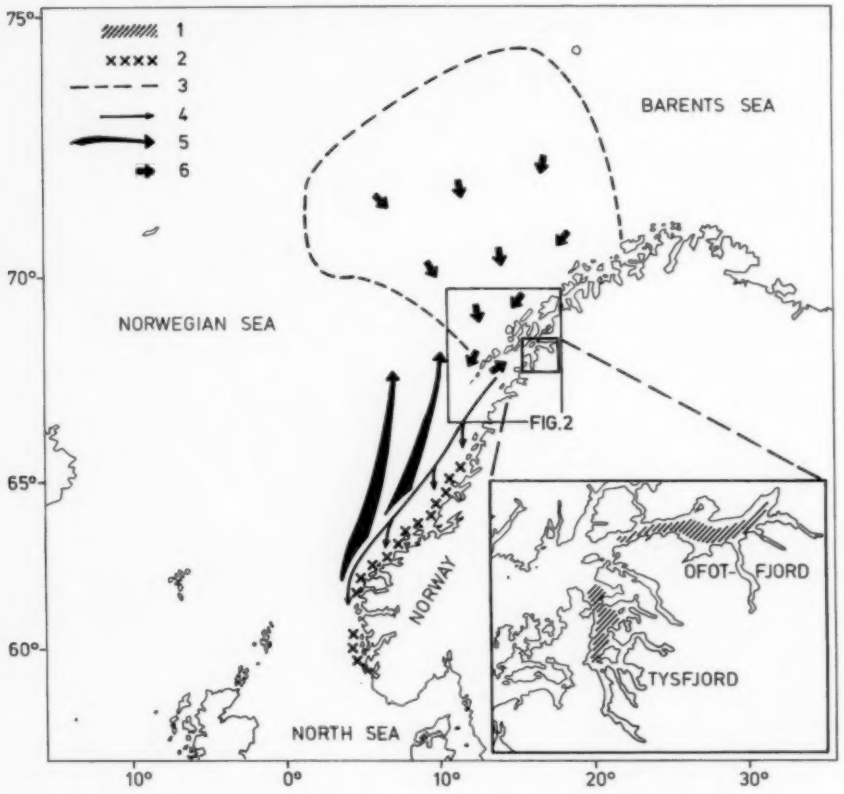
Fig. 1. The approximate yearly migration pattern of the adult fraction of the Norwegian spring-spawning herring stock during 1990–1993. 1, wintering area; 2, spawning area; 3, feeding area; 4, spawning migration; 5, feeding migration; 6, wintering migration. The area marked Fig. 2 covers the killer whale study area.



and killer whales occur in October to January in the Lofoten area (wintering grounds of herring) and in February and March off the coast of Møre (the spawning grounds of herring) (Christensen 1988; Øien 1988; Hamre 1990; Røttingen 1990). Lyrholm (1988) reported movements of photoidentified individual killer whales between Lofoten and Møre. The Lofoten area is the only area in Norwegian coastal waters where killer whales have been reported year-around (Christensen 1988).

The Norwegian spring-spawning herring performs large-scale seasonal migrations between the wintering, spawning, and feeding areas in Norwegian coastal waters and in the Norwegian Sea (Dragesund et al. 1980; Hamre 1990; Røttingen 1990) (Fig. 1). Following the major stock collapse in the late 1960s (Dragesund et al. 1980), the migratory pattern of the herring changed from oceanic to more coastal, and since 1987 the wintering area has been mainly in two fjords, Tysfjord and Ofotfjord, in northern Norway (Hamre 1990; Røttingen 1990; International Council for the Exploration of the Sea 1994) (Fig. 1). The concentration of the wintering grounds to a small area in coastal waters has offered a unique possibility for intensive studies on herring and on one of its predators, killer whales.

We present results from a 4-year (1990–1993) photoidentification study of killer whales in waters around the islands of Lofoten and Vesterålen in northern Norway (Fig. 1). Our main objective was to examine fall–winter and summer occurrence of photoidentified killer whale pods (groups) in relationship to the distribution of Norwegian spring-spawning herring. Furthermore, potential pod-specific differences in occurrence or diet were investigated.

Material and methods

Study area and periods of fieldwork

The study area included the waters around Lofoten and Vesterålen islands and the Tysfjord–Ofotfjord area in northern Norway (Fig. 1). In a pilot study, photographs of killer whales were taken in this area in 1983–1986 (Lyrholm 1988) and in 1987–1989 (T. Similä, unpublished data). On the basis of the experience from the pilot study, core study areas were identified within the overall study area for potential occurrence of killer whales during different seasons (Fig. 2).

In the summers of 1990–1991 there were two core areas off the Vesterålen islands (V1 and V2), and in 1992–1993 an additional core area was found around the islands of Røst and Værøy in Lofoten (L). In fall–winter (1990 –1993) one large core study area was divided into five subareas: one in Vestfjord (VF), one in Vestfjord–Ofotfjord (OF), and three in Tysfjord (TF1, TF2, TF3). Two of these subareas

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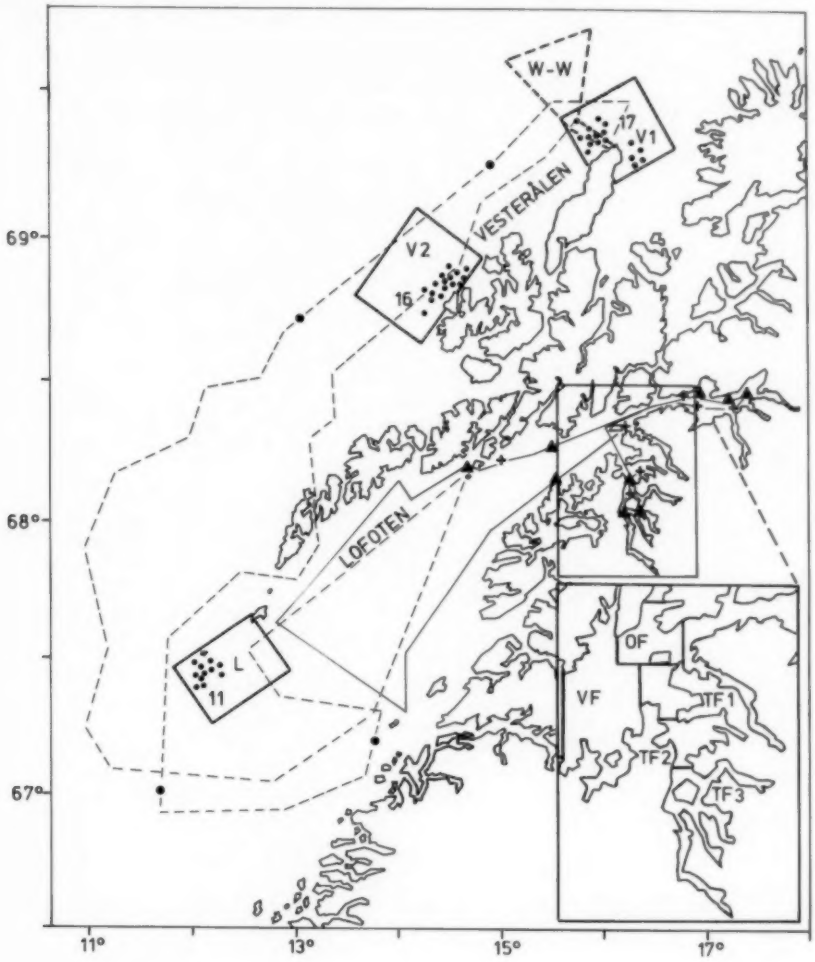
Fig. 2. Killer whale sighting surveys and locations of pod encounters in the summer core study areas V1, V2, and L. The solid line marks the sighting surveys on November 18–27 in 1990 and 1991, and the broken line marks the sighting survey on August 15–24 in 1992. ▲, areas where killer whales were sighted in November 1990; +, areas where killer whales were sighted in November 1991; ●, areas where killer whales were sighted in August 1992. The symbols VF, OF, and TF13 mark the fall–winter core study areas. W–W, the area where sperm whale watching trips operated during June–August.



(TF1, TF2) were visited more often than the others. Fieldwork was done in October to November and June to August and in 1991–1992 also in January to February (Table 1).

Methods used in obtaining observations of killer whales

Killer whales were searched using two different methods. (*i*) During the 300 days of fieldwork in the core study areas, we searched for killer whales by using a combination of land watch, telephone calls to pilot stations, ferries, and fishing boats and by searching in the area with the research vessel. (*ii*) Ten-day sighting surveys were conducted once in November 1990 and once in November 1991 on board RV *Johan Ruud* and in August 1992 on board MS *Leif Junior* (Fig. 2). The November sighting surveys covered the fall–winter core study areas (VF, OF, TF1–TF3) as well as Ofotfjord and a major part of Vestfjord. The summer sighting survey was done in coastal and offshore waters off the Lofoten and Vesterålen islands, including all three summer core areas (V1, V2, L). During sighting surveys whales were observed from crow's nests or flying bridges from vessels cruising along predetermined transect lines.

Methods used in estimating relative occurrence of killer whales

Relative occurrence of killer whales during October–November and January–February 1991–1992 was calculated on the basis of killer whale encounters in the core areas VF, OF, TF1-3 (not including data from sighting surveys). A killer whale encounter was an occasion where one or more individuals in a killer whale pod were photoidentified. The occurrence was calculated from ED/EFD where effort day (EFD) is a day spent searching for whales and encounter day (ED) is a day whales were encountered (one encounter per day regardless of the number of pods encountered). The number of pods encountered

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Table 1. Dates and areas of killer whale fieldwork in the core study areas in 1990–1993 and the boats used.

Date	Area	Boat
1990		
June 8–13	V1	MS *Spekkulf* (9.3 m)
June 25–27	V1	MS *Spekkulf*
July 22–25	V2	MS *Spekkulf*
Aug. 1–2	V2	MS *Spekkulf*
Aug. 14–18	V1	MS *Spekkulf*
Sept. 9	V1	Zodiak (3.5 m)
Oct. 16 – Nov. 15	TF, OF, VF	MS *Spekkulf*
1991		
Jan. 15.1 Feb.15	TF, OF, VF	MS *Spekkulf*
June 11–15	V2	MS *Spekkulf*
July 18	V1	Zodiak
July 23	V1	Zodiak
July 25	V1	Zodiak
July 31	V1	Zodiak
Oct. 8 – Nov. 17	TF, OF, VF	MS *Spekkulf*
1992		
Jan. 5 – Feb. 3	TF, OF, VF	MS *Spekkulf*
Feb. 4–6	TF, OF, VF	MS *Øyprinsen* (13.5 m)
June 8–15	L	MS *Øyprinsen*
June 25 – July 4	V2	MS *Spekkulf*
July 10	V1	MS *Spekkulf*
July 19–21	V1	MS *Spekkulf*
Oct. 7 – Nov. 20	TF, OF, VF	MS *Spekkulf*
1993		
June 14–24	L	Zodiak
June 28 – July 7	V2	SY *Oda* (11.5 m)
July 15–19	L	MS *Øyprinsen*
July 14, 20, 21	VF	MS *Øyprinsen*
Oct. 13 – Nov. 18	TF, OF, VF	MS *Sandøy* (9.3 m)

Note: For the location of the core study areas, see Fig. 2.

per effort day (PEC/EFD) was also calculated for each season. Boat traffic in the fall study area increased from 1990 to 1993 (research vessels and whale-watching vessels searching for killer whales) and the assistance in finding the killer whales increased correspondingly.

During the summer months, killer whales were sought in the core study areas (L, V1, and V2) with variations in the methods used and areas covered, and therefore the data were not used for estimating relative occurrence. A more consistent data set consisted of sighting data received from whale-watching vessels, which operated outside Andenes, Vesterålen, in June–August. The whale-watching vessels took out tourists daily, weather permitting, for 5- to 8-h trips to see sperm whales (*Physeter macrocephalus*) in an area overlapping the core study area V1 (Fig. 2). All sightings of killer whales made during these trips were recorded.

Photoidentification of killer whales

Photographs taken of killer whales were used for studying the seasonal occurrence of different killer whale pods (groups). Killer whales were identified by natural markings on the dorsal fin and the grey saddle patch behind the fin (Bigg et al. 1990). The pictures were taken with 35-mm SLR cameras using 300-mm lenses and 400 ASA black and white film pushed to 1600 ASA. The films were analyzed for identifications with a stereoscopic microscope. Identification pictures were taken during sighting surveys, during field studies in the core areas, and occasionally from the whale-watching vessels.

Pod is a term used for groups of killer whales with stable membership (Bigg et al. 1990). In the study area, killer whales occur in groups that seem to have a stable structure (T. Similä and F. Ugarte, unpublished data). The analysis of seasonal occurrence of killer whales was not based on sightings and resightings of individuals but of pods (all individuals of a pod were assumed to be present even if only a few individuals were identified). This approach was chosen because there are substantial differences in the resightability of killer whale individuals (owing to differences in the recognizability of individuals) (Similä and Lindblom 1993), whereas the resightability of killer whale pods is more equal (well-marked individuals are present in all pods).

Each of the killer whale pods was given an alphabetic code, e.g., NA, where N is for Norway and A is the name of the pod. Once the capital letters from A to Ø had been used, the same letters were used again in lower case.

Killer whales were also photographed at the coast of Møre (Bisther and Vongraven 1995). Photographs from these two areas were compared for analyzing movements of killer whales between the spawning and wintering grounds of herring.

Species identification of killer whale prey

Prey species eaten by killer whales were identified during encounters with feeding whales by observing either the prey species or pieces of it at the surface. During clear-water conditions in the fall in 1991–1993, an underwater video camera with a view of the upper 15 m of the water column was used to observe feeding behavior (Similä and Ugarte 1993). When possible, the age of herring was determined as adult (≥29 cm) or adolescent (<29 cm) on the basis of length measurements from herring captured during the feeding bouts.

Distribution and abundance of herring

The distribution and abundance of Norwegian spring-spawning herring was monitored regularly in 1990–1993 by the Institute of Marine Research (IMR), Bergen, Norway, as part of the routine stock assessment programmes. The wintering area in Ofotfjord and Tysfjord was surveyed yearly in November–December and January, while the feeding areas off the Vesterålen and Lofoten islands and in the Norwegian Sea were surveyed in July–August 1991 and 1993.

Continuous echointegration of fish registrations was carried out with a Simrad EK-500 echointegrator and the abundance of herring was estimated according to the method described by Midtun and Nakken (1971) and Dalen and Nakken (1983). Samples of herring were obtained with a standard pelagic trawl hauled at about 4 kn (1 kn = 1.852 km/h) at depths that varied according to the observed concentrations of herring. Samples of up to 100 fish were taken randomly from the catches and individual fish were weighed, their total length was measured, and various parameters such as sex, maturation stage, and age were recorded.

Most of the juvenile stock was distributed outside the study area during the period investigated, so only interactions between killer whales and adolescent and adult herring will be considered.

Results

Seasonal occurrence of killer whales

The majority of killer whale pod encounters were made in October and November in Vestfjord, Tysfjord, and Ofotfjord (core areas VF, OF, TF1–3) (Fig. 3). Most encounters were made in the two subareas (TF1, TF2) that were surveyed more often than the others. In October and November 1990–1992, killer whales were encountered on all effort days in the core study area (Table 2). In 1992, both the total number of pods identified and the number of pod encounters per effort day were higher than during the other years. In the fall of 1993, killer whales were not encountered on all effort days, and the number of different pods encountered was lower than in

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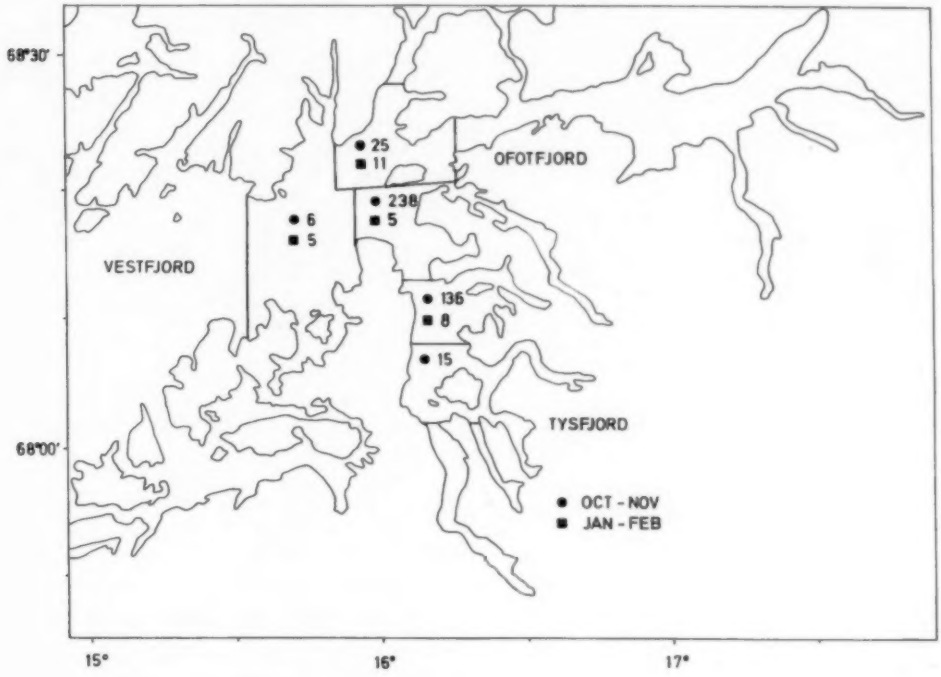
Fig. 3. Locations of killer whale encounters in the fall–winter core study area (not including sighting surveys) during October and November 1990–1993 and January and February 1991–1992. The numbers represent the amount of pod encounters in the subareas of the core study area.



1990–1992 (Table 2). A total of 420 killer whale pod encounters were made in October and November 1990–1993 (data from sighting surveys not included) and 20–28 different killer whale pods were identified each fall.

During the sighting surveys in late November 1990 and 1991, killer whale pods were sighted within the core study area and in the Ofotfjord (Fig. 2). In 1990, 18 pods were sighted and 10 of these were identified; in 1991, 10 pods were sighted and 6 of these were identified. The pods identified in Ofotfjord (seven encounters) were all pods that had also been encountered in the core study area.

Killer whales started leaving the Tysfjord–Ofotfjord area during the first weeks of January, and both the encounters per effort day and the number of different pods identified were lower in January and February than in October and November (Table 2). A total of 29 pod encounters were made, and six different pods were identified in January and February 1991–1992. In 1991, the last killer whale encounter was made on February 11 and in 1992 on January 22.

In June–August, killer whales were encountered in all of the summer core study areas (L, V1, V2) in waters around the Vesterålen islands and around the outermost islands of Lofoten (Fig. 2). A total of 44 killer whale pod encounters were made in the core study areas in June–August, and each summer 4–9 different pods were identified. During the sighting survey in August 1992, killer whales were sighted throughout the study area (Fig. 2). Six killer whale pods were sighted and four of them were identified; one pod was encountered twice. The occurrence of killer whales varied between the summers;

Table 2. Relative occurrence of killer whales during October and November 1990–1993 and January and February 1991–1992.

Year	EFD	ED	ED/EFD	PEC	PEC/EFD	PODS
October–November						
1990	23	23	1.00	68	2.96	26
1991	32	32	1.00	127	3.97	27
1992	35	35	1.00	153	4.37	28
1993	24	22	0.92	72	3.00	20
January–February						
1991	22	14	0.63	20	0.91	5
1992	14	7	0.50	9	0.64	4

Note: EFD, effort day, day spent searching for whales; ED, encounter day, day killer whales were encountered; PEC, pod encounter; PODS, number of different pods encountered.

killer whales were sighted during 5–18% of the whale-watching trips (Table 3). In addition to sperm and killer whales, harbor porpoises (*Phocoena phocoena*) and minke whales (*Balaenoptera acutorostrata*) were often sighted from the whale-watching vessels.

Patterns in occurrence of photoidentified killer whale pods

A total of 408 killer whale individuals were identified in the study area in 1990–1993. The whales were provisionally divided into 39 different pods (Table 4). The sizes of the pods varied between 6 and 30 individuals, with a median size of 15 individuals.

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Table 3. Sightings of killer whales made during sperm whale watching trips outside Andenes, Vesterålen, in June–August 1990–1993.

Year	Trips	Sightings	% of trips
1990	96	12	12
1991	128	11	9
1992	132	24	18
1993	154	8	5

Note: A sighting of killer whales was registered as one observation, regardless of the numbers of killer whales seen.

During the 4 years of study, 525 pod encounters were made (Table 4). Generally, the same pods were seen in the study area each year (Table 4). The number of resightings was high in 1991–1993, and the cumulative number of pods identified (27, 32, 38, and 39 in 1990, 1991, 1992, and 1993, respectively) suggests that the majority of killer whale pods occurring in the area have been identified. There were differences in the frequency of encounters of the different pods (Table 4; Fig.4); 81% of the pods were encountered 1–20 times during the study period. Seven pods (NA, NC, NE3, NG30, NO, NP, NY) were encountered 26–47 times and they made up 47% of all encounters. These seven pods and eight others (NB, NE15, NG11, NQ, NT, NV, NW, NÅ) were encountered in each of the study years, but only NP was seen each season and year. Each year there were several resightings of the seven most often encountered pods, except for 1992, when NE3 and NO were encountered only a few times (Table 4).

A total of 34 different pods were encountered during October–February and 23 different pods in June–August. Eighteen pods (47%) were encountered during both fall–winter and summer, and five pods (10%) were encountered only during summer (Table 4). Sixteen pods (42%) were encountered only during fall–winter (Table 4) and one of these, NN3, was seen only in the late winter when most of the other pods had left the study area.

Killer whales belonging to seven different pods (NA, NC, NG30, NP, NQ, NU, and KA) were identified both in the study area and in the spawning grounds of herring at the Møre coast (Lyrholm 1988; D. Vongraven, Institute of Zoology, University of Trondheim, 7055 Dragvoll, Norway, personal communication).

Prey species identified

The methods used in identifying prey were most suitable for detecting killer whales feeding on schooling fish, marine birds, or marine mammals near the surface and were less likely to detect solitary fish or squid being eaten.

In 99 out of the 105 occasions when the prey among feeding killer whales was identified, the prey was herring. However, most observations of feeding killer whales were made in October and November; herring was identified as prey on 89 occasions in the fall but only on 10 occasions in the summer. The pods observed feeding on herring during the summer were NA, NE5, NG30, NO, NP (three observations), NT, NØ, and NÆ.

The length of the herring preyed upon by killer whales was estimated on 35 occasions (Fig. 5). Adult herring dominated the diet in the fall (94%), whereas adolescent herring dominated the samples collected in the winter (100%) and the summer (70%).

In October 1993, a killer whale was observed eating a saithe (*Pollachius virens*) that was swimming close to a herring school on which the killer whale and its pod were feeding. In June 1990, a killer whale pod fed on mackerel. In October 1991, a group of unidentified killer whales were seen feeding on eider ducks (*Somateria molissima*). In October 1992, a juvenile from NW pod ate a little auk (*Alle alle*), and in July 1992 a juvenile from NP pod ate a northern fulmar (*Fulmarus glacialis*). The chasing of eider ducks appeared to be organized hunting in which several birds were taken, whereas the feeding on the two other birds looked like playing behavior. Once a young killer whale was observed eating a jellyfish, and the whales were often observed playing with jellyfishes. The three species of seabirds and jellyfish, mackerel, and saithe are all additions to the list of known prey of Norwegian killer whales.

In June 1993, the NØ pod was observed trying to catch a young harbor seal (*Phoca vitulina*) in Røst, Lofoten, without succeeding. On the following day, the same whales fed on herring in the same area.

Seasonal distribution and migratory pattern of herring

The adult part of the herring stock showed a rather stable migratory pattern throughout the period studied. At the end of the oceanic feeding season, the herring concentrated in large schools off the Vesterålen and Lofoten islands in late August, when herring schools also entered the Vestfjord basin. The herring concentrated in the two main wintering fjords, Tysfjord and Ofotfjord (an area covering approximately 680 km^2), from early October and remained in the area until about mid-January. The adult herring (which comprised 96–98% of the biomass) started the southbound spawning migration by mid-January (Fig. 1) while the adolescent herring remained in the fjords a bit longer. The estimated biomass of herring in the wintering area remained fairly stable throughout the period studied, with estimates ranging from about 2.2 to 3.4 million tonnes (International Council for the Exploration of the Sea 1993, 1994). Besides herring, cod and saithe were present in the trawl hauls in the wintering area of herring.

Throughout the period studied, the spawning grounds were situated between Egersund and Vesterålen, although the main spawning grounds were observed off Møre (Fig. 1). After the spawning season the herring started a northwestern migration in March and April towards the Norwegian Sea feeding area. The main feeding area was observed off Vesterålen and southern Troms, out to about 0° west, and from 68 to 75° north, in an area covering approximately 250 000 – 300 000 km^2 in July–August. A southwestward extension of the feeding area took place during 1991 to 1994 (International Council for the Exploration of the Sea 1995). In general, the adolescent herring fed closer to shore than the adult herring that were observed further west and north. Besides herring, significant concentrations of mackerel, horse mackerel (*Draculus draco*), lumpsucker (*Cyclopterus lumpus*), and saithe were present in the trawl catches within, or close to, the killer whale core study area during the summer.

Besides this general migratory pattern, immature herring originating from the Barents Sea enter the areas off Vesterålen and Troms, where they feed for 1 or 2 years before entering

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Table 4. Killer whale pod encounters during 1990–1993 in October and November (F) and June to August (S).

	1990		1991		1992		1993		Total		
Pod	F	S	F	S	F	S	F	S	F	S	Sum
					Both seasons						
NA	8	—	10	—	8	—	4	4	30	4	34
NB	6	—	7	—	4	—	1	2	18	2	20
NE3	4	2	7	—	1	1	8	3	20	6	26
NE5	2	3	6		6	—	—		14	3	17
NE15	6	—	5	1	2	—	6	—	19	1	20
NG11	5	—	1	—	4	—	2	1	12	1	13
NG30	6	—	13	—	18	2	4	2	41	4	45
NL	4	—	1	1		—			5	1	6
NN17	5	—			1	1	1	1	7	2	9
NO	5	2	5	—	4	—	10	—	24	2	26
NP	2	3	10	5	12	4	4	2	28	14	42
NT	3	—		1	1	8	—	2	14	1	15
NU			3	—	4	—	—	1	7	1	8
NY	4	1	7	—	9	1	4	—	24	2	26
NÅ	2	—	1	—	4	1	2	—	9	1	10
NÆ					1	—	—	1	1	1	2
Nb	1	—			1	1			2	1	3
Nc			1	—	1	—	3	1	5	1	6
					Fall only						
NC	6	—	15	—	20	—	6	—	47	—	47
ND	2	—							2	—	2
NG8	6	—	3	—	3	—			12	—	12
NN3	9	—	3	—					12	—	12
NQ	3	—	4	—	10	—	1	—	18	—	18
NR	1	—	2	—	1	—			4	—	4
NS	1	—							1	—	1
NV	1	—	6	—	3	—	3	—	13	—	13
NW	1	—	7	—	5	—	4	—	17	—	17
NX			3	—	3	—			6	—	6
NZ			4	—	10	—	1	—	15	—	15
KA			8	—	5	—	5	—	18	—	18
Na	2	—	4	—					6	—	6
Ne					4	—			4	—	4
Nf	3	—	5	—			1	—	9	—	9
Ng					1	—			1	—	1
					Summer only						
NK	—	1	—	1	—	1			—	3	3
NG58					—	2			—	2	2
NØ					—	1	—	3	—	4	4
Nd					—	1	—	1	—	2	2
Nh							—	1	—	1	1
Totals											
No. enc.	98	13	142	9	153	16	72	23	465	61	525
No. pods	27		28		32		25				
Resightings			23		26		24				

Note: Fall 1990 includes January and February 1991, and fall 1991 includes January and February 1992. The data include all killer whale pod encounters (i.e., pods identified in the core study areas, during sighting surveys, and from the whale-watching boats).

the spawning stock. During the period studied, the 1988 and 1989 year-classes left the Barents Sea and recruited to the spawning stock from 1992. Most of these herring adopted the migratory pattern of the older herring, although some of the 1989 year-class was observed spawning off Vesterålen in March and April 1993.

Discussion

Seasonal occurrence and diet of killer whales

Earlier studies demonstrated a connection between the occurrence of killer whales and herring off Lofoten and Vesterålen islands in northern Norway during the wintering period for

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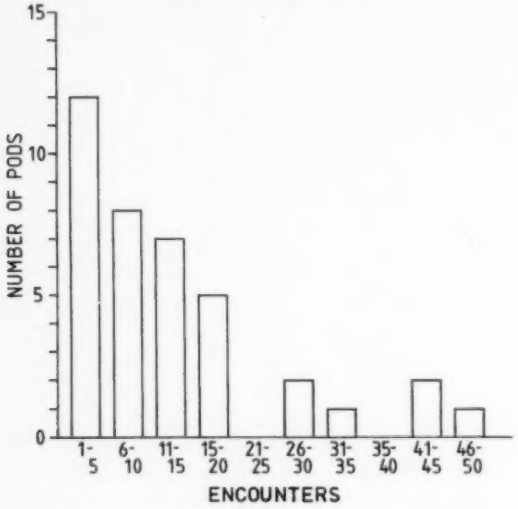
Fig. 4. Frequency distribution of encounters of the different killer whale pods photoidentified in northern Norway in 1990–1993. The data include all occasions when killer whale pods were identified in the core study areas during the sighting surveys and from the whale-watching boats in Andenes.



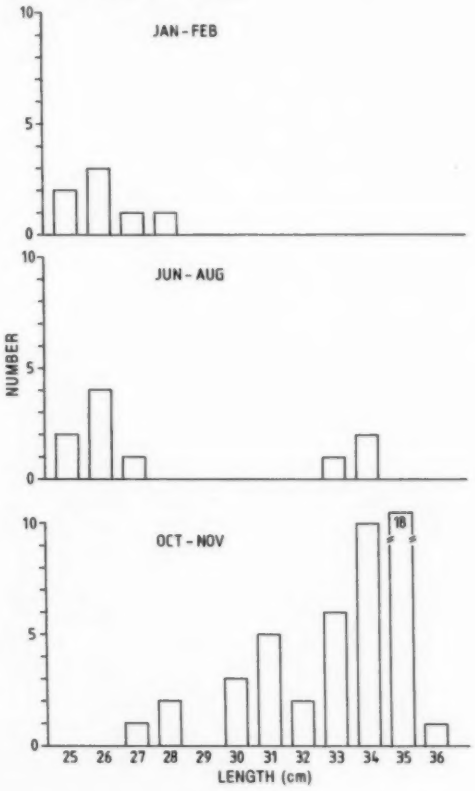
herring (Christensen 1988; Øien 1988). However, the identity of the killer whales occurring in these waters and their areas of occurrence and diet during summer months were not known.

This study shows that it was mainly the same killer whale pods that returned to the study area in northern Norway year after year; almost half of the pods were observed yearly and the number of resightings was high in 1991–1993. The cumulative number of pods identified suggests that the majority of killer whale pods occurring in the study area was identified.

There was a consistent pattern in the seasonal occurrence of killer whales in the study area: the whales occurred in different areas during October to January and June to August and were encountered most often in October and November. In the fall, killer whales occurred in the Tysfjord–Ofotfjord area, which was the wintering area for the whole adult stock and part of the adolescent stock of the spring-spawning herring. The adult herring dominated both the biomass of wintering herring and the diet of killer whales in October and November. Cod and saithe were present in the wintering area of herring and could have contributed to the diet of the killer whales, as indicated by the one observation of saithe being eaten by a killer whale in Tysfjord and by cod being found in the stomach contents of killer whales caught in the Lofoten area in winter (Christensen 1982).

The increase in the number of pod encounters from fall 1990 to 1992 could be related to an increased searching effort. However, in October and November 1993, the searching effort was considerably higher than in previous years, but fewer killer whale encounters were made. The estimated abundance of herring in the wintering area remained stable from 1992 to 1993, so diminished food resources do not explain this decrease in killer whale encounters. As this was a period of strong herring recruitment, it is possible that some killer whale

Fig. 5. The length distribution of herring caught among feeding killer whales in January and February (n = 7, caught during five different feeding occasions from January 20 to February 11, 1991), June to August (n = 10, caught during eight different feeding occasions in 1990–1993), and October and November (n = 48, caught during 22 different feeding occasions in 1990–1993). Adolescent herring are <29 cm, and adult herring are ≥29 cm long.



pods exploited the alternative resources of recruiting adolescent herring that wintered outside the study area.

Killer whales left the Tysfjord–Ofotfjord area in early winter when the adult herring started to migrate to the spawning grounds. The onset of the herring spawning migration was relatively abrupt, with almost complete desertion of the wintering area within a few weeks around mid-January. In winter 1992, no killer whale pods were observed after the 3rd week of January. In 1991, killer whales feeding on the adolescent herring still present in the fjords were encountered until the 2nd week of February.

During the summer, killer whales occurred off the Lofoten and Vesterålen islands and this area overlapped with the herring feeding area. Mostly adolescent herring occurred in these nearshore waters and dominated the few observations of killer whale diet. Mackerel and saithe were abundant in the summer study area and could have been included in the diet of killer whales to a larger degree than indicated by the one observation of killer whales feeding on mackerel.

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The results of this study suggest that marine birds are not an important part of the diet of killer whales, either during the fall or in the summer. The unsuccessful attempt of killer whales to catch a young harbor seal was the only indication of killer whales feeding on marine mammals, although harbor seals, harbor porpoises, sperm whales, and minke whales were present in the summer study area and are known to be part of the diet of killer whales elsewhere (Jefferson et al. 1991).

During the summer months, herring fed in small schools over an area of several thousand square kilometres, which could explain the irregular occurrence of killer whales and the small number of different pods observed each summer in the coastal waters studied. According to Øien (1993), offshore waters in the Norwegian Sea are an important habitat for killer whales during the summer months and more observations of killer whales could probably have been obtained by doing surveys further off the coast.

Of the seven killer whale pods encountered in both the wintering and spawning grounds of herring, four (NA, NP, NG30, and NC (in 1989)) were also observed in the summer feeding grounds of herring, which indicates that at least these pods follow the main migration route of the Norwegian spring-spawning herring stock throughout the year.

In British Columbia and Washington state, the resident killer whales follow summer salmon runs to the coastal waters around Vancouver Island (Heimlich-Boran 1988; Nichol 1990). The locations and timing of these salmon runs have been generally the same over a long period of time, and the resident killer whales are highly familiar with their environment and have developed location-specific behavioral traditions (Heimlich-Boran 1988; Felleman et al. 1991). The size and the migration pattern of the Norwegian spring-spawning herring stock have varied considerably, often with abrupt changes in summer and winter distribution (Devold 1963; Dragesund et al. 1980; Røttingen 1990), and these changes seem to have been followed by killer whales (Christensen 1988; Øien 1988). Therefore, long-term seasonal occurrence of killer whales in the coastal waters of northern Norway is unpredictable and location-specific traditions are not likely to develop. The herring stock is expected to grow rapidly in the coming years (International Council for the Exploration of the Sea 1994), and as a consequence, major changes could be expected in the present seasonal distribution pattern of both herring and killer whales.

Differences in occurrence of killer whale pods

The stable social structure within killer whale populations creates the potential to form populations with distinct social and behavioral characteristics. Off British Columbia and Washington state, three different types of killer whales occur: transients, residents, and offshore killer whales (Bigg et al. 1990; Felleman et al. 1991; Ford et al. 1994). The transient killer whales have a wide home range, occur irregularly, and feed mainly on marine mammals. Resident killer whales occur regularly and feed on salmon (*Oncorhynchus* spp.) and other fish. Less is known about the offshore killer whales, but they probably feed on herring and other fish. Because of these findings and similar results from other parts of the world (Berzin and Vladimirov 1983; Ellis 1987), it was of interest to investigate if any differences could be found in the pattern of seasonal occurrence or diet of the killer whale pods occurring in northern Norway.

The majority of killer whale pods identified in this study seemed to share a similar pattern of occurrence and was encountered during both the fall and the summer. After each summer of field studies, more pods were added to the category observed during both seasons, and it is possible that all pods observed in the wintering area of herring may occur in the study area during the summer.

The seven most commonly encountered pods (NA, NC, NE3, NG30, NO, NP, and NY) could have been more stationary in the study area or simply favoured the two most often surveyed subareas of the fall study area (TF1 and TF2). In British Columbia, the resident killer whale pods show area preferences within their general summer home range (Ford et al. 1994).

The five pods (NG58, NK, NØ, Nd, and Nh) that were observed only during the summer may have a different pattern of seasonal occurrence and prey preference than the pods encountered in the wintering area of herring. It is interesting that the six pods identified in the area prior to 1990 (T. Similä, unpublished data) and not resighted during 1990–1993 were all encountered during the summer. When all of the pods identified in 1983–1993 are considered, as many as 24% (11 pods) were observed only during the summer. The single pod (NØ) observed only during the summer that was also observed feeding was trying to catch a young harbor seal in June 1993 and was seen feeding on herring the following day in the same area.

One of the pods (NN3) was encountered several times during both winter surveys but not earlier in the wintering season of herring. This pod was present when the adult herring and almost all other pods had left the study area and was exploiting adolescent herring still present in the fjords. The pattern of occurrence of this pod is difficult to explain by changes in the herring abundance because it entered the study area when herring biomass was starting to diminish.

Killer whale social groups and populations are known to differ from each other in their vocal repertoire (Ford 1991). In a comparison of call repertoires of 10 Norwegian and 4 Icelandic killer whale pods, Strager (1995) found that the NN3 pod was acoustically quite different from the other Norwegian pods and shared a call with Icelandic whales. Since the call is complex in structure, it is unlikely to have evolved independently in the two areas (Strager 1995). Jonsgård and Lyshoel (1970) suggested that killer whales might have moved between Icelandic and Norwegian waters during the earlier (prior to 1970) migration pattern of the Norwegian spring-spawning herring. The NN3 pod might represent a present or previous contact between the two areas.

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Seasonal patterns of metal accumulation in Arctic char (*Salvelinus alpinus*) from an oligotrophic Alpine lake related to temperature

G. Köck, M. Triendl, and R. Hofer

Abstract: Cd and Pb concentrations in the liver, kidney, and stomach contents of Arctic char (*Salvelinus alpinus*) from an oligotrophic high mountain lake (Schwarzsee ob Sölden Tyrol, Austria) with low pH (4.8 to 6.4) and alkalinity (–8 to +8 μequiv.·L^{-1}) were investigated over the course of 1 year. Peak concentrations of Cd and Pb in the water coincided with the drop of pH during snowmelt, whereas metal concentrations in liver and kidney were lowest at the end of winter and increased during summer. Furthermore, the Cd and Pb load of the food was considerably higher in winter than during summer. Thus, fluctuations in the metal levels in fish did not correlate with seasonal variations in the metal concentrations in lake water or in the diet. Rather, patterns of metal accumulation in the fish indicated that enhanced metal uptake was a consequence of increasing metabolic rates during summer. Thus, water temperature appeared to be the driving force of metal accumulation in fish from an oligotrophic lake. Concentrations of Cd and Pb in liver and kidney were positively correlated to age. A simple empirical model illustrates the ability of the fish to eliminate considerable amounts of accumulated metals.

Résumé : On a étudié pendant 1 an les concentrations de Cd et de Pb dans le foie, les reins et le contenu de l'estomac d'ombles chevaliers (*Salvelinus alpinus*) provenant d'un lac oligotrophe de montagne situé en altitude (Schwarzsee ob Sölden Tyrol, Autriche), dont l'eau présentait un faible pH (4,8 à 6,4) et une faible alcalinité (–8 à +8 μéquiv.·L^{-1}. Les concentrations maximales de Cd et de Pb dans l'eau coïncidaient avec la chute du pH pendant la fonte nivale, tandis que les concentrations d'espèces métalliques dans le foie et les reins étaient au minimum à la fin de l'hiver et augmentaient durant l'été. De plus, la charge de Cd et de Pb dans les aliments était considérablement plus élevée à l'hiver qu'à l'été. Il n'y avait pas de corrélation entre ces fluctuations de concentrations d'espèces métalliques dans les poissons et les variations saisonnières des concentrations de métaux dans l'eau du lac ou dans la nourriture. Les modes d'accumulation de métaux dans le poisson indiquaient plutôt que c'était l'accroissement des taux métaboliques durant l'été qui était à l'origine de l'absorption accrue de métaux. La température de l'eau serait donc responsable, semble-t-il, de l'accumulation d'espèces métalliques dans les poissons des lacs oligotrophes. Il y avait une corrélation positive entre l'âge et les concentrations de Cd et de Pb dans le foie et les reins. Un simple modèle empirique permet de montrer que les poissons peuvent éliminer des quantités considérables de métaux accumulés.
[Traduit par la Rédaction]

Introduction

In freshwater fish, metal uptake and toxicity are largely influenced by the pH, Ca concentration, and alkalinity of the water (Alabaster and Lloyd 1980; Spry and Wiener 1991). On one hand, under acidic conditions the free divalent ion is the dominant form of many metals, readily absorbed by fish gills (Merlini and Pozzi 1977; Pärt et al. 1985). On the other hand, interference of H^+ ions with metal uptake at the gill surface has been proposed as the dominant mechanism for decreasing metal toxicity at acid pH (Pärt et al. 1985; Cusimano et al. 1986). Competition between Ca^{2+} or Mg^{2+} and divalent metal ions for binding sites on the gill surface and Ca^{2+}-mediated changes in the permeability of gill membranes lead to diminished metal accumulation and toxicity in hard waters (Calamari et al. 1980; Pagenkopf 1983; Verbost et al. 1987; Wicklund and Runn 1988). Recently we have shown the Cd and Pb concentrations in the kidney of Arctic char (*Salvelinus alpinus*) from oligotrophic Austrian mountain lakes to be negatively correlated to alkalinity (Köck et al. 1995); despite low Cd and Pb concentrations in the water (Cd, 0.1 μg·L^{-1}; Pb, 0.5 μg·L^{-1}, dissolved), metal concentrations in the kidneys of char from the most acidic study lake (Schwarzsee ob Sölden) were extremely high and comparable with those of fish from metal-polluted waters. Furthermore, we observed indications of seasonal effects on metal concentrations in fish.

The aim of the present study was to investigate metal accumulation in fish from a single lake as a complement to the relationship among lakes previously established (Köck et al. 1995). We investigated the seasonal pattern, and elucidated the causes, of metal accumulation in Arctic char from an acidic lake.

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G. Köck,[1] M. Triendl, and R. Hofer. Institute of Zoology and Limnology, University of Innsbruck, Technikerstrasse 25, A-6020 Innsbruck, Austria.

[1] Author to whom all correspondence should be addressed. e-mail: Guenter.Koeck@uibk.ac.at

Material and methods

Schwarzsee ob Sölden (SOS; 46°57′57″N, 10°56′46″E) is a high-altitude lake located in the Oetztaler Alps in Northern Tyrol (Austria) at 2799 m above sea level. The lake has a surface area of 3.5 ha, a maximum depth of 18 m, and a catchment area of only 18 ha, and it

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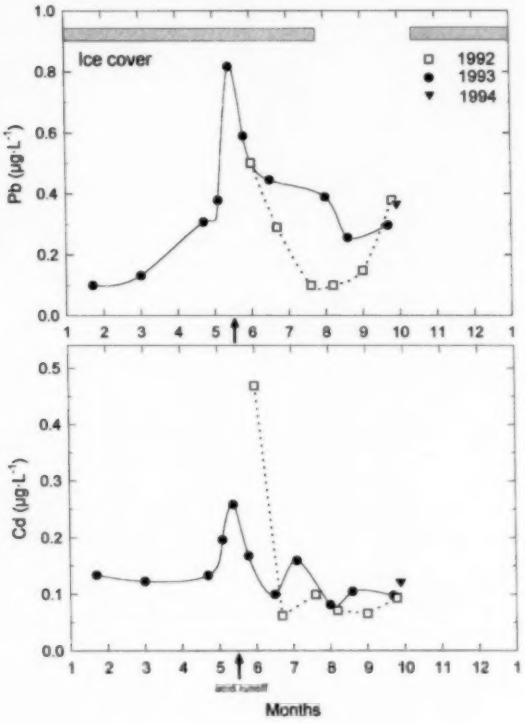
Fig. 1. Concentration of dissolved Pb and Cd in SOS water (volume-weighted mean values).



is ice covered for more than 9 months. The bedrock of the catchment consists of granitic gneisses of the Oetztal crystalline.

The water chemistry data of the lake were reported in detail in Köck et al. (1995). In brief, SOS is an oligotrophic lake with low alkalinity (−8 to +8 µequiv.·L^{-1}) and low pH (4.8 to 6.4). Na and Cl concentrations are 0.25 ± 0.03 and 0.13 ± 0.03 mg·L^{-1}, respectively. As far as we know, SOS is the highest fish-bearing lake in the Alps, and information on fish status spans several decades.

Arctic char (*Salvelinus alpinus*) were caught (n = 88) with gill nets of 12.5-, 16-, 19-, 22-, 25-, and 30-mm mesh size in summer and with hook and line during the ice-covered period. Owing to the oligotrophic character of SOS the growth performance of Arctic char is poor. Biometric data of fish are as follows: length, 18–28 cm; body mass, 49–195 g; condition factor (calculated according to the formula $CF = 100\ W/L^3$, where W is body weight in grams and L is total body length in centimetres), 0.63–0.99; sex ratio, 48 females to 40 males. Ages of the fish (determined from otoliths and (or) opercula) ranged from 4 to 16 years with 25% of the fish being older than 10 years.

Samples of liver, kidney, and stomach contents were digested by addition of HNO_3 and H_2O_2 in a microwave oven. Water samples from different depths (from surface to bottom in 2-m intervals; volume-weighted averaging) were collected by a polyacrylic Schindler sampler and acidified for preservation. Metals were analyzed by graphite furnace atomic absorption spectrophotometry (GFAAS) with Zeeman background correction. Details of sampling procedures, metal analysis, and quality assurance protocols are described in Köck et al. (1995). Water temperature and pH were measured at different depths biweekly during ice break and during the ice-free period and monthly during the ice-covered period.

Macroinvertebrates were collected with an Ekman sampler and hand netted from the water surface and in the shallow littoral zone of the lake. Invertebrates from the field and samples of stomach contents obtained after dissection of fish in the laboratory were preserved in 70% ethanol for subsequent identification. The proportion of different invertebrate taxa in the entire food spectrum was estimated volumetrically (Hyslop 1980). Owing to their high digestibility the proportion of oligochaetes in stomach contents might be underestimated.

Metal data are given on a dry weight basis and can be transformed to wet weight by multiplying by 0.25 ± 0.03 (liver) and 0.19 ± 0.02 (kidney).

Data were tested by one-way analysis of variance (ANOVA) followed by a Bonferroni t test for multiple comparisons versus a control group (ice-break group: mid July). Regression analysis was used to test age-dependent metal accumulation in fish tissues. Data were corrected for age by analysis of covariance to ensure comparability between the sampling dates. Metal data for males and females were compared using Student's t test. Statistical significance was assigned at $p \leq 0.05$.

Results

Analyses of lake water

The concentrations of Pb and Cd in SOS lake water were low and showed relatively small interannual variation during the observed period (Fig. 1). Concentrations of both Pb and Cd showed a peak during May. We observed a simultaneous drop in the pH, indicating the input of atmospherically deposited acids and metals during snowmelt (acid runoff; Fig. 2). Water temperature increased after ice break from a constant level of 1.5–2°C during the ice-covered period to a maximum of 8–10°C in late August (Fig. 2). Depth profiles of temperature (Fig. 2, inset) indicate that water temperature increased with increasing depth during the ice-covered period and the pattern was reversed in summer.

Metal analyses of fish samples

Regression analysis revealed significant age-specific effects on the metal burden in liver and kidney. The concentrations of Pb and Cd in liver (R = 0.55 and 0.60 for Pb and Cd, respectively) and kidney (R = 0.51 and 0.72 for Pb and Cd, respectively) were correlated positively to the age of fish ($p < 0.01$). Because no significant differences in metal concentrations were found between the sexes, data from males and females were combined.

Results of ANOVA and the Bonferroni t test indicated significant seasonal effects on Pb and Cd concentrations in liver and kidney (Fig. 3). In the liver, metal concentrations reached their lowest seasonal level immediately before ice break; after ice break, however, metal concentrations increased rapidly. The increase in tissue metal concentrations during summer coincided with the increase in water temperature after ice break. A similar pattern could be found in the kidney but with the increase in metal concentration commencing a few weeks later than in the liver. Concentrations of both Cd and Pb in liver and kidney were significantly higher at the end of summer than during winter and spring. In contrast to Pb, concentrations of Cd in the kidney decreased significantly from May to the beginning of August and increased until the end of summer.

Analysis of stomach contents revealed a marked seasonal change in the main components of the diet (Fig. 4) and

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Fig. 2. Seasonal variation of temperature (at 5 m depth) and pH (at 1.5 m depth). The insert shows the depth profiles of temperature and pH at different sampling dates.

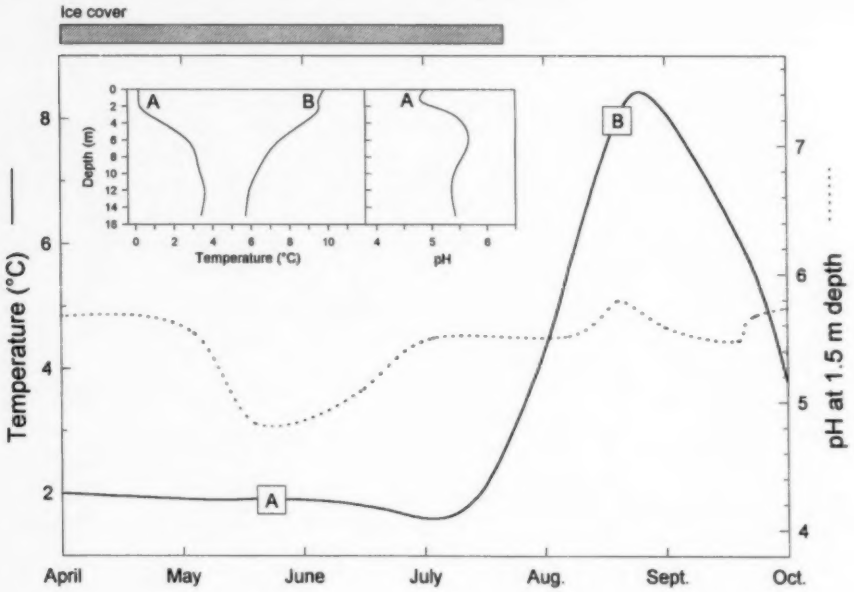

Fig. 3. Seasonal variation of metal concentrations in liver and kidney of Arctic char from SOS. Means ± SE (dry weight) and number of fish (in parentheses) are given. The broken line shows the seasonal variation in the water temperature at 5 m depth. *, significantly different from the ice-break group (shown by the diamond in mid July).

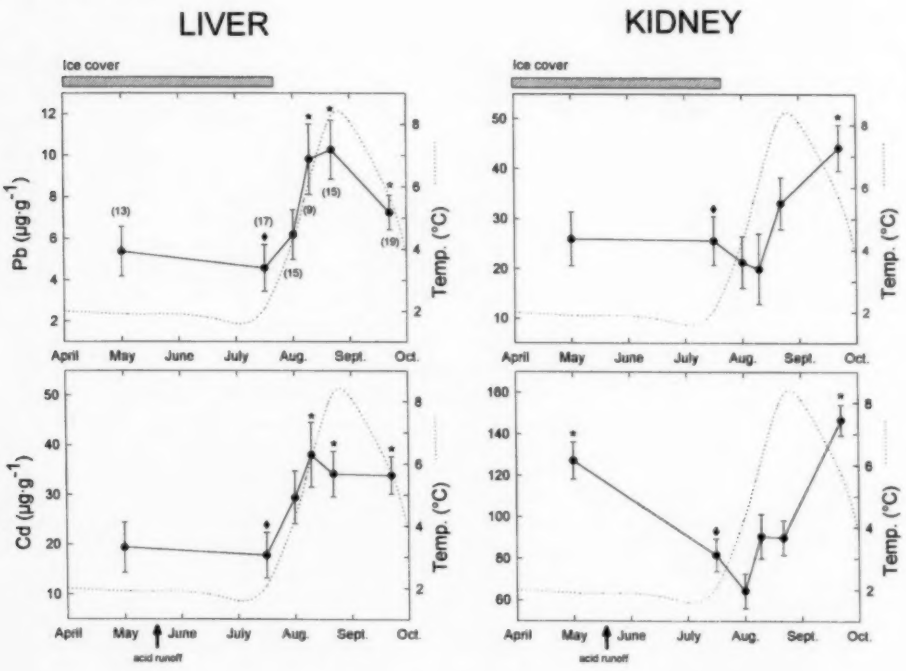

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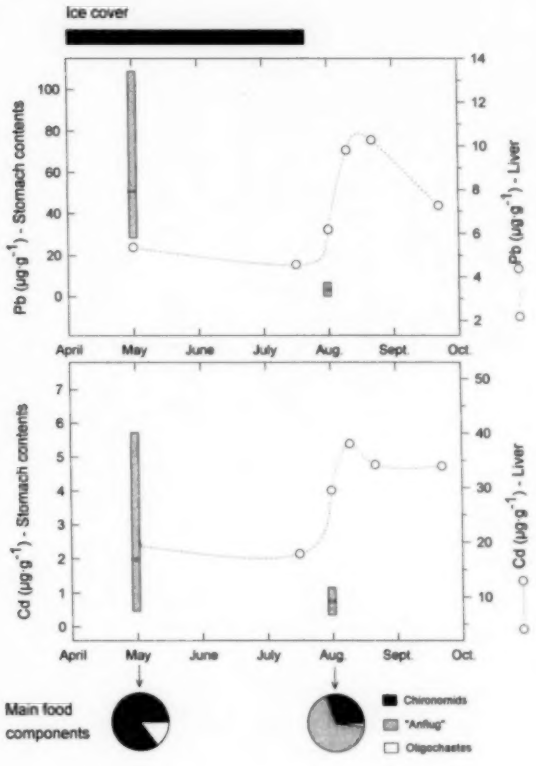
Fig. 4. Seasonal variation in the metal concentration in stomach contents (dry weight) of Arctic char from SOS. Bars represent median and percentiles (25 and 75%). Pie slices show the main components of food (%). The seasonal variation in the metal concentration in the liver (see Fig. 3) has been added (open symbols and broken line).



reflected the level of Cd and Pb contamination of the invertebrate samples from the field (oligochaetes > chironomids ⩾ allochthonous prey "Anflug"; Triendl 1994): in winter 85% of the prey consumed were chironomid larvae. In summer, however, stomach contents were dominated by flying insects (67%) with low levels of metal contamination ingested from the water surface ("Anflug"). Concentrations of Pb and Cd in stomach contents were much higher in winter than in summer.

Discussion

Because Cd and Pb concentrations in liver and kidney were found to be extremely high (Fig. 3) and comparable with those of fish from waters receiving metal-polluted industrial effluents (Köck et al. 1995), the question about the source of metals arises. It is well known that meltwater concentrates atmospherically deposited acids and pollutants from the snowpack, resulting in a temporary acidification of surface waters and a pulsed input of toxicants (Johannessen and Henriksen 1978; Jefferies et al. 1979; Franzin 1984). Maximum concentrations of Cd and Pb in SOS water during May coincided with a marked decrease in pH (acid runoff; Figs. 1 and 2). Because the drop in lake pH was more pronounced in the upper layers of the water column, remobilization of metals from the sediment may be of minor significance (Fig. 2). Thus, atmospheric deposition seems to be the most likely source of metals in SOS water.

A number of factors may contribute singly or in combination to seasonally changing metal accumulation in fish, namely sexual differences and variation in the water temperature and (or) metal concentration in the water and in the diet (Dallinger et al. 1987; National Research Council of Canada 1979; Nicoletto and Hendricks 1988). Our results clearly show that annual cycles of pH, metal concentration in the water, and metal load of the diet play only a minor role in the seasonal pattern of metal concentration in liver and kidney of Arctic char from SOS. The metal load of the food is considerably higher in winter (autochthonous prey) than during summer (allochthonous prey). Furthermore, concentrations of Cd and Pb in the water attain their maxima during May. Metal concentrations in the liver, however, were lowest at the end of winter and increased during summer. Obviously, the progressive increase in the metal burden in the liver coincides with the period of rising temperatures during summer. Temperature is an important factor controlling metabolism in poikilothermic animals (Fry 1971; Hochachka and Somero 1971; Schmidt-Nielsen 1979). Many studies have shown an increase in temperature to be associated with enhanced toxicity of metals to fish (Cairns et al. 1975; Hodson and Sprague 1975; Roch and Maly 1979; McGeachy and Dixon 1989). Fewer data, however, are available for temperature-dependent accumulation of trace metals, although accumulation of mercury in fish is widely recognized to be positively correlated to water temperature (MacLeod and Pessah 1973; Reinert et al. 1974; Hoffmann 1987; Bodaly et al. 1993). The mechanisms by which temperature modifies accumulation and toxicity of metals are not yet fully understood. Rising water temperatures result in increasing gill ventilation rates owing to higher oxygen demand for metabolic requirement and (or) decreasing oxygen concentration in the water (Heath 1987; Niimi 1987; Douben 1990). This leads to a higher volume of water passing the gills and increased uptake of metals from the water. During winter, elimination exceeds the uptake of metals, leading to decreasing metal concentrations in liver and kidney. Although Pb and Cd concentrations in the liver decrease in autumn (significant for Pb only), when temperature declines, concentrations remain significantly higher than at the ice break (Fig. 3). Since the regulation of these nonessential elements is less effective than that of essential trace metals (like Zn and Cu), it is assumed that fish are not able to eliminate Pb and Cd completely. This would lead to a year to year accumulation of Cd and Pb in a stepwise manner (Fig. 5). In the char population studied, a positive correlation exists between age and both Cd and Pb concentration in liver and kidney, indicating an accumulation of Cd and Pb over the entire life-span of the fish. On the basis of this correlation we established a simple empirical model of annual metal turnover in fish (Fig. 5). The hypothetical annual increase in Pb concentration in the liver, as calculated by regression analysis of age-dependent Pb burden in the liver, amounts to 0.8 µg Pb·g^{-1} (dry weight). Although both uptake and elimination of metals take place throughout the year, our data show that the main portion of the annual uptake occurs during summer. Setting the accumulation rate

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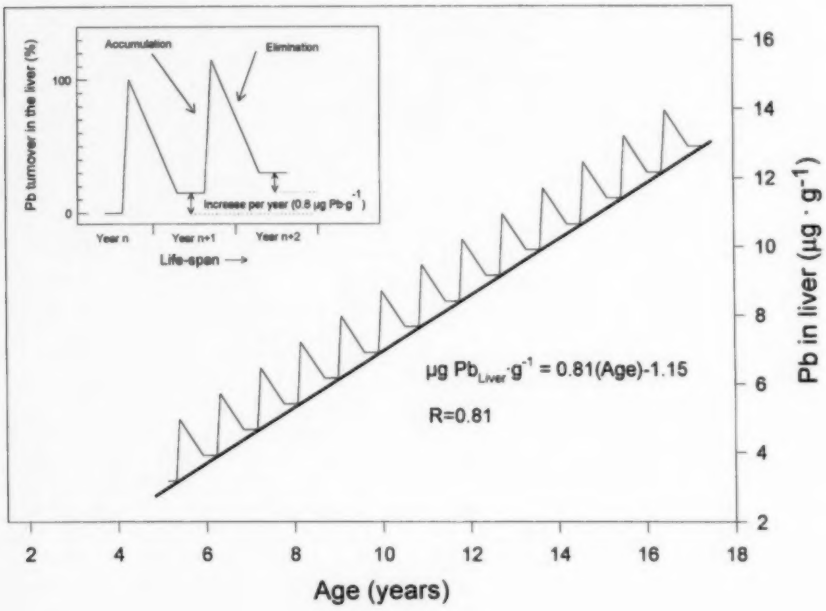
Fig. 5. Model of annual Pb turnover in liver of Arctic char from SOS. The model is based on regression analysis of age-dependent Pb burden in the liver (88 fish) and Pb accumulation data derived over the course of 1 year. The accumulation rate during summer was set as 100% of annual metal uptake rate.



during summer as 100%, a fish would have to eliminate more than 80% of accumulated Pb to explain the calculated net uptake of 0.81 µg Pb·g^{-1} (dry weight) per year. Calculated elimination rates for Pb in the kidney and Cd in liver and kidney compare well with that of Pb in the liver (80–90%).

Reproductive cycles do not appear to be the cause of the observed elevated tissue concentrations. On the other hand, water temperature and photoperiod are the most important environmental cues for reproductive functions, with temperature being the dominant one (Heath 1987; Lam 1983). In SOS char the increase in metal concentrations in the liver coincides with the period of gonadal development. Owing to the synthesis of large amounts of vitellogenin in the liver, the metabolic activity of female fish is high (Olsson et al. 1987; Povlsen et al. 1990). Metallothionein (MT) plays a major role in metal regulation in fish. Its main functions are detoxification of nonessential metals like Cd and Hg and maintenance of the homeostasis of Zn and Cu (Roesijadi 1992). In fish, hepatic MT levels fluctuate as a function of the reproductive status, possibly in response to changing metabolic requirements for Zn and Cu, since these elements are essential cofactors of many enzymes involved in the sythesis of DNA, RNA, and proteins (Olsson et al 1987; Overnell et al. 1987). The increase in the MT level in female fish is much larger than in males (George and Langston 1994). Since the affinity of MT for Cd is higher than for Zn, a combined increase in Cd with de novo synthesized MT might be possible. In our study, however, the increase in metal concentrations in the liver of male fish is as high as in female fish. Furthermore, levels of Pb, which is not sequestered by MT, increased during the same period. Thus, we assume that the increase in metal levels during summer is not a consequence of newly induced MT during gonadal development but a result of higher uptake rates owing to increasing water temperature.

In the kidney, levels of Pb and Cd are much higher than in the liver, indicating the kidney to be a main target for metals (Fig. 3). The decrease in metal concentrations during spring and early summer indicates a depuration phase. Since depuration of Cd from fish tissues has been shown in several studies (Muramoto 1980; Oronsaye 1987; Douben 1989; Harrison and Klaverkamp 1989; Handy 1992), a route for Cd excretion must exist in fish. However, literature findings about the possible role of the kidney in metal excretion are contradictory (Kumada et al. 1973; Giles 1988; Wicklund Glynn et al. 1992). In mammals Cd is excreted through the kidney but urinary Cd excretion has been shown to play a minor role in fish (Giles 1988; Roesijadi 1992). Some authors report loss of metals via the bile and gut (Grahl et al. 1985; Giles 1988; Harrison and Klaverkamp 1989); others suggest metal excretion via gills, skin, or body mucus (Varanasi and Markey 1978; Oladimeji et al. 1984; Oronsaye and Brafield 1984; Heath 1987). In fish from SOS the observed depuration phase continued during the period when metal concentrations in the liver had already begun to increase (Fig. 3). The increase in metal concentrations in the kidney occurred some weeks later than in the liver. Thus, we assume that in contrast to the situation in the liver, the metabolism of metals in the kidney is not directly related to ambient temperature.

In summary, our study illustrates the ability of Arctic char to eliminate considerable amounts of accumulated metals. Fluctuations in the metal levels in fish from SOS do not correlate with seasonal variations in the metal concentrations in

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the food and in lake water. Patterns of metal accumulation in fish from SOS indicate enhanced metal uptake to be a consequence of increasing metabolic rates during summer. Thus, water temperature appears to be the driving force of metal accumulation in fish from oligotrophic lakes.

Acknowledgements

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Variability in survival of larval fish: disentangling components with a generalized individual-based model

Benjamin H. Letcher, James A. Rice, Larry B. Crowder, and Kenneth A. Rose

Abstract: Many factors, including intrinsic characteristics of the fish themselves and extrinsic factors of the biological environment, have the potential to regulate mortality rates during the early life of fishes. We used a detailed simulation model to rank the effects of variability in these factors on larval and early juvenile survival. Our major finding was that proportional changes in the intrinsic and extrinsic factors in the model had equal effects on cohort survival. Of the intrinsic factors, growth capacity (metabolism and assimilation efficiency), not foraging ability or starvation resistance, explained the most variance in survival. Of the extrinsic factors, predator size explained 83% of the variability in survival but proportional changes prey availability had only a minor effect. Variability in prey density required a 3-fold increase to equal the effects of predator size on survival. Despite the important effects of predation pressure on survival, it had only a minor impact on how fish died. Whether fish died from predation or starvation depended much more on the intrinsic variables related to metabolism and starvation resistance and on the density of the smallest prey type.

Résumé : De nombreux facteurs, dont les caractéristiques intrinsèques des poissons et les facteurs extrinsèques de l'environnement biologique, peuvent réguler les taux de mortalité au cours des premiers stades de vie des poissons. Nous avons utilisé un modèle permettant de faire des simulations détaillées pour classer les effets de la variabilité de ces facteurs sur la survie des larves et des très jeunes poissons. Le fait que des changements proportionnels dans les facteurs intrinsèques et extrinsèques du modèle aient des effets égaux sur le taux de survie de la cohorte constituait notre principale constatation. Parmi les facteurs intrinsèques, c'est la capacité de croissance (efficacité du métabolisme et de l'assimilation), et non la capacité de s'alimenter ou la résistance au manque de nourriture, qui expliquait la plus grande partie de la variance du taux de survie. Parmi les facteurs extrinsèques, la taille des prédateurs permettait de rendre compte de 83% de la variabilité dans le taux de survie, mais des changements proportionnels dans la disponibilité des proies n'avaient qu'un effet mineur. Dans le cas de la variabilité dans la densité des proies, il fallait une augmentation par un facteur de trois pour obtenir un effet égal aux effets de la taille des prédateurs sur la survie. Malgré ses effets importants sur la survie, la pression de prédation n'avait qu'un impact mineur sur la façon dont mourait le poisson. La raison de la mort du poisson, c'est-à-dire prédation ou manque de nourriture, dépendait beaucoup plus des variables intrinsèques liées au métabolisme et à la résistance au manque de nourriture, ainsi que de la densité du plus petit type de proies.
[Traduit par la Rédaction]

Introduction

Mortality in the early life of fishes is typically very high, and small variations in these rates can result in widely varying cohort survival and subsequent recruitment (Sissenwine 1984; Houde 1987; Bailey and Houde 1989; Beyer 1989). Because of the variability in mortality rates and the large number of factors that modify them, survival to a particular age, size, or stage (i.e., recruitment) is difficult to understand and to predict. Despite an extensive search for stock-recruit relationships, simple descriptive models do not provide adequate predictions of year-class strength. An alternative and possibly more fruitful approach to understanding mortality rates is to examine the mechanisms underlying component processes and their interactions (May 1974; Fogarty 1993).

Many previous efforts to understand the causes of mortality have focused on single processes: a critical period during which food limitation will cause massive starvation mortality (Hjort 1914), the match–mismatch of the larval period with abundant food resources (Cushing 1975), the distribution of food in the water column (Lasker 1975, 1978), oceanographic transport and retention mechanisms (Hjort 1914; Parrish et al.

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B.H. Letcher,[1] **J.A. Rice, and L.B. Crowder.**[2] Department of Zoology, Box 7617, North Carolina State University, Raleigh, NC 27695-7617, U.S.A.
K.A. Rose. Environmental Sciences Division, P.O. Box 2008, Oak Ridge National Laboratory, Oak Ridge, TN 37831-6036, U.S.A.

[1] Author to whom all correspondence should be sent at the following address: National Biological Service, S.O. Conte Anadromous Fish Research Center, One Migratory Way, P.O. Box 796, Turners Falls, MA 01376, U.S.A.
[2] Present address: Duke University School of the Environment, Marine Laboratory, 135 Duke Road, Beaufort, NC 28516-9721, U.S.A.

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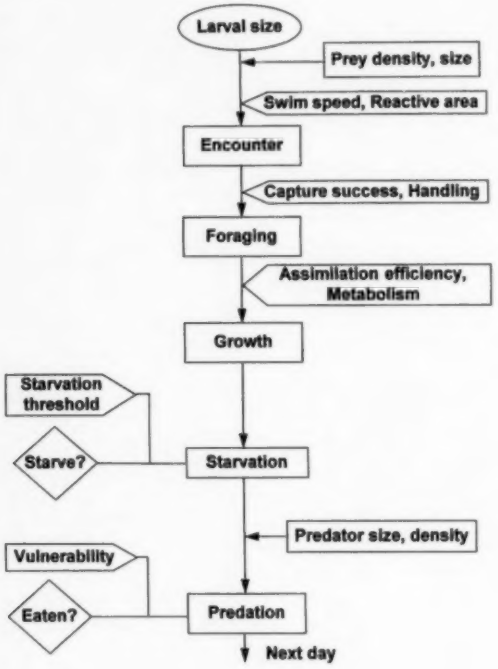
Fig. 1. Flow diagram of the model. All functions in the model depend on larval size. The five submodels determine encounter, foraging, growth, starvation, and predation rates. The major processes affecting each submodel are in the pointed boxes. Characteristics of the larvae's environment (food and predators) are in the boxes with horizontal arrows. The model calculates growth rates for each larva every day of the simulation. Larvae could die from either predation or starvation. Each simulation started with 7500 larvae 3.69 mm long.



1981; Sinclair 1988), and predation (Cushing 1974; Hunter 1981, 1984; Sissenwine 1984; Bailey and Houde 1989). However, all of these processes may interact (Fritz et al. 1990), and one or several may be important at a particular time or place. Also, further complicating predictions, particular species (Houde 1987) or even individuals (Uchmanski 1985; Lomnicki 1988) may react differently to environmental conditions. Clearly, many factors including food, predators, physical processes, and differences in species or individual morphology, physiology, or behavior can influence survival of young fishes. All of these are very difficult to measure synoptically in the field and would require a massive factorial experimental design to adequately capture the dynamics in a laboratory or in mesocosms. Simulation modeling is an alternative that provides a context for evaluating the effects of many interacting factors.

We designed a detailed individual-based simulation model to compare the effects on survival of extrinsic environmental factors (food and predators) with those attributable to intrinsic characteristics of fish (foraging and bioenergetics). Individual-based models (IBMs) have become popular recently (Huston et al. 1988; DeAngelis and Gross 1992; Van Winkle et al. 1993) because they represent individuals and local interactions explicitly. Variability among individuals in foraging ability (Magurran 1986; Marschall et al. 1989) and growth (Rubenstein 1981; Uchmanski 1985; Lomnicki 1988) is common among fishes. This variability, which can result in a wide range of sizes for any age (Uchmanski 1985) and different survival probabilities for individual fish (Sharp 1987; Rice et al. 1993), can be represented easily with IBMs. IBMs can also accommodate variability in the fish's environment. In this model, we include both the prey and predators of the fish larvae, the two external factors that can directly determine survival via starvation and predation.

In addition to mortality rates, we also evaluated how variation in intrinsic and extrinsic factors affects whether fish die from predation or starvation. Much work on recruitment and larval fish has focused on sources of mortality, but the relative importance of predation and starvation mortality seems to vary greatly with species and habitat. Vulnerability to both predation and starvation generally decreases for larger fish but which is likely to be more important as a cohort of fish grows? We used the IBM to explore whether it is possible to discern any general patterns in the relative importance of starvation and predation mortality as a function of variation in intrinsic and extrinsic factors.

Because of the data limitations and because our goal was to explore general patterns in survival of young fishes, we sacrificed precision for generality (Levins 1966) and created a detailed but generalized model of larval fish; it depicts individuals in an averaged planktonic fish population and so does not necessarily describe any one species or habitat, but it should provide useful insights for larval fish in general by indicating directions, magnitudes, and relative effects. To make a general model we need a way to summarize data from various species. While each species of fish is ultimately unique, many of the processes important to survival of larval fish are size dependent (Anderson 1988; Miller et al. 1988; Beyer 1989). In the model presented here, all functions are size dependent. We drew these functions from the literature, using existing functions, or deriving our own when necessary. In our literature survey, we focused primarily on pelagic species with planktonic larvae.

Despite the large number of existing IBMs for fish, none has provided an extensive sensitivity analysis. Sensitivity analysis yields an estimate of the relative importance of model components. Most results presented in this paper are from sensitivity analyses. Ranking effects of model variables can be used to identify key processes, reduces the complexity of detailed models such as IBMs, and focuses future hypotheses, indicating which processes we may be able to ignore and which we may need to explore in more detail.

Model description

General

The model simulated the feeding, growth, and survival of individual fish larvae. Each fish was defined by its length, weight, and age and could die from either predation or starvation. To simulate growth and mortality of the larvae, every fish passed through a series of steps each day (Fig. 1). These steps included the major processes that affect the early life of fish

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Table 1. Nominal length, mass, and density of the four prey types used in the model.

	Prey no.	Length (mm)	Mass (μg)	Density (no./mL)	*a*	*b*	Reference
Rotifer	1	0.20	0.182	0.200	1.845	1.44	Dumont et al. 1975
Nauplius	2	0.25	0.282	0.075	3.009	1.706	Culver et al. 1985
Copepodite	3	0.50	1.410	0.005	4.592	1.703	Culver et al. 1985
Copepod	4	0.83	4.988	0.0005	7.700	2.33	Dumont et al. 1975

Note: Mass was calculated from length using length–weight relations from the literature where mass = $a \cdot \text{length}^b$.

(Hunter 1981; Blaxter 1986; Bailey and Houde 1989) and were divided into five submodels: (*i*) prey encounter to estimate encounter rates of larvae with their prey, (*ii*) foraging to determine food consumption rates, (*iii*) bioenergetics–growth to convert food consumed into growth, (*iv*) size-dependent predation risk to calculate probabilities of mortality from predators on the larvae, and (*v*) starvation to define when fish would starve. Each submodel depended on a set of processes or variables (pointed boxes in Fig. 1) described in detail below. The processes in the growth and starvation submodels were functions of fish mass, while those in the prey encounter, foraging, and predation submodels were primarily functions of larval length (except capture success of feeding larvae).

We derived function parameter values in one of four ways: using functions from previous review articles (e.g., swimming speed in Miller et al. 1988), compiling and summarizing data from the literature (e.g., routine metabolic rate), using relationships directly from the literature when data were available for only one species (e.g., reactive distance as a function of fish and prey size), and estimating reasonable values when data were not available (e.g., larval capture success on non-naupliar prey). When data were compiled from different sources in which the experiments were performed at different temperatures, data were standardized to a common temperature (15°C). Although temperature will affect many of the processes in the model directly (e.g., metabolism), we did not explicitly include temperature in the model because the indirect effects of temperature on the population dynamics of larval fish prey and predators are complex and unknown and because sufficient data on how temperature influences the details of the interaction between predators and prey are not available.

In the model, the larvae's environment consisted of prey for the larvae and predators on the larvae (Fig. 1). Prey were randomly distributed in space on an intermediate scale (10s of metres) but they were aggregated into patches on a larger scale (100s of meters and 3 times more dense than nonpatches, see Letcher and Rice (1996) for more details). Thus, the prey environment of the fish consisted of patches through which the fish could swim on a daily time scale. Because the larvae were planktonic and in fairly large bodies of well-mixed water (100s of metres, well mixed within patches and outside patches), we assumed that prey densities were constant, i.e., the larvae did not affect their prey (see also Laurence 1982; Cushing 1983); this assumption is reasonable given the typically low average densities of larvae relative to their prey. We chose four prey types representing the range of typical larval fish prey (rotifer, copepod nauplius, copepodite, and copepod (Table 1)). Prey lengths and weights were estimated from the literature (Table 1). Prey densities were set at levels that decreased by about an order of magnitude with each increase in prey size-class (Hunter 1981; MacKenzie et al. 1990; range 0.0005–0.2/mL, Table 1) and were initially set such that they yielded maximum daily consumption rates (defined below) for all fish sizes in the model. This allowed us to express prey density as a proportion of the larvae's maximum consumption.

Predators were cruising and gape limited and were defined by length and density. Starvation was mass dependent: if a larva lost a certain proportion of its previous maximum body mass, it died from starvation.

Each model simulation started with 7500 newly hatched larvae (25 μg dry weight, 3.69 mm total length (TL), about the median hatch size of 66 species in Miller et al. (1988, their Fig. 1)). Throughout the model, we used a composite length (ℓ, mm) – weight (W, μg dry) relationship derived from 13 species of freshwater and marine larvae (*Archosargus rhomboidalis* (Stepien 1976), *Coregonus clupeaformis* (Taylor and Freeburg 1984), *Stenotonus chrysops* (Laurence 1979), *Pseudopleuronectes americanus* (Laurence 1979), *Melanogrammus aeglefinus* (Laurence 1979), *Menidia beryllina* (Letcher and Bengtson 1993*a*), *Paralichthys dentatus* (Laurence 1979), *Engraulis mordax* (Lasker et al. 1970; Theilacker 1987), *Gadus morhua* (Laurence 1979), *Theragra chalcogramma* (Yamashita and Bailey 1989), *Limanda ferruginea* (Laurence 1979), *Engraulis encrasicolus* (Regner 1983)):

$$(1) \quad W = \text{LWInt} \cdot \ell^{\text{LWExp}}$$

with parameters LWInt and LWExp. Parameter values for this and all other equations are in Table 2. During model runs, length and mass were uncoupled; fish did not lose length, but they could lose weight. Growth was added as mass, and when lengths exceeded the value defined by eq. 1 for a given mass, lengths were updated from the new mass. Larvae did not grow in length or weight until the day of first feeding (FF), defined as a function of length (Miller et al. 1988):

$$(2) \quad \text{FF} = 4.09 - \text{FFSlope} \cdot \ell$$

Larvae were vulnerable to predation before first feeding. From eq. 2, day 3 was the day of first feeding for fish in the model (3.69 mm hatch size).

Prey encounter submodel

Encounter rate

Encounter rates of larvae with their prey were functions both of fish size and prey size and of prey density (Blaxter 1986). Encounter rates are the product of search volume and prey density and were computed separately for each of the four prey types. We defined encounter rate (ER, number/s) with each prey type as

$$(3) \quad \text{ER}_{\ell,i} = \text{SV}_{\ell,i} \cdot \rho_i \cdot \text{Light}$$

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Table 2. Nominal variable and parameter values with short descriptions.

	Variable or parameter name	Nominal value	Equation no.
Initial number of fish	InitNum*	7500	—
Average initial mass (μg dry)	AvgStart*	25	—
CV of initial size	CVStart*	0	—
Length–weight relationship intercept	LWInt*	0.1674	1
Length–weight relationship exponent	LWExp*	3.837	1
Prey length 1–4 (mm)	PyLen(1–4)*	See Table 1	—
Prey mass 1–4 (μg dry)	PyMass(1–4)*	See Table 1	—
Prey density 1–4 (no./mL)	PyDen(1–4)*	See Table 1	—
Average prey density (prop. C_{max})	AvgPreyDen	0.64	—
Predator length (mm)	PredSize	50	21
Predator density (no./L)	PredDen	1×10^{-12}	20
Prop. of day available for feeding	Light*	13/24	3,16,28
First feeding function slope	FFSlope*	0.237	2
Swimming speed intercept	SSInt*	0.776	5
Swimming speed exponent	SSExp*	1.07	5
Prop. of reactive area used for feeding	Prop*	0.5	6
Handling time slope	HTSl*	7.0151	10
Handling time intercept	HTInt*	0.264	10
Capture success numerator (prey types 1–4)	CSNum(1–4)*	0.95; 0.90; 0.70; 0.90	Fig. 2
Capture success denominator (prey types 1–4)	CSDen(1–4)*	10.0; 750.0; 5×10^{7}; 5×10^{8}	Fig. 2
C_{max} function intercept	CmaxInt*	2.8275	13
C_{max} function exponent	CmaxExp*	0.8496	13
Maximum assimilation efficiency	AssimMax*	0.8	15
Assimilation efficiency shape parameter	AssimSh*	0.002	15
Routine metabolism numerator	MetabNum*	4500	17
Routine metabolism denominator	MetabDen*	45 000	17
Specific dynamic action + egestion	SDA+E*	0.3	16,28
Activity metabolism multiplier	ActMetab*	2.5	16,28
Starvation threshold	Thresh*	0.75	18,25,26
Predator's reactive distance multiplier	PredRadM*	0.8	19
Predator's swimming speed multiplier	PredVSS*	3.0	20
Predator's capture success exponent	CaptExp*	2.28	21
Predator's capture success numerator	CaptNum*	3.37	21
Predator's capture success denominator	CaptDen*	44.76	21
Proportion of encounters that predator attacks	PropAtak*	0.5	22
Slope of the 50% mortality function	M50Sl*	0.801	24

Note: Food for the larvae are referred to by prey numbers (1–4, Table 1). Equation numbers indicate the location of each parameter in the text. Variable names followed by an asterisk were used in the individual parameter perturbation.

where SV was search volume (mL), ρ_i was the density of prey type i (number/mL, Table 1), and Light equaled the proportion of the day (13 h/24 h) during which there was sufficient light for feeding. Light level was a constant and did not influence feeding rate. Encounter rates increased rapidly and monotonically with larger fish size but decreased with larger prey size because smaller prey had higher densities. We calculated actual encounter rates from a random distribution (Poisson) with mean and variance equal to ER.

Search volume

Search volume (SV, mm^3/s) was the volume of water in which fish perceive prey and was defined as the product of the fish's swimming speed (SS) and the area of water a fish of length ℓ can see (RA, see Blaxter 1986):

$$(4) \quad SV_{\ell,i} = SS_{\ell} \cdot RA_{\ell,i}$$

Swimming speeds for different species as a function of fish length are highly variable. We used the summary relationship of Miller et al. (1988, their Fig. 6*a*) to provide a length-dependent estimate of average swimming speed (SS, mm/s)

$$(5) \quad SS_{\ell} = SSInt \cdot \ell^{SSExp}$$

where SSInt and SSExp were parameters. This equation yields swimming speeds of approximately 1 body length/s.

Reactive area (RA, mm^2) was a half circle with radius equal to reactive distance and was defined for each prey type i as

$$(6) \quad RA_{\ell,i} = (RD_{\ell,i})^2 \cdot \pi \cdot Prop$$

where Prop = 0.5 and accounted for the assumption that larval fish actually perceive one half of the circle defined by reactive distance (RD) (Blaxter 1986). Search volumes increase rapidly with increases in fish size and prey size.

We defined RD (mm) as a function of both prey length (PL_i, mm, for each prey type i, Table 1) and larval length (ℓ, mm) using the relationship of Breck and Gitter (1983). This

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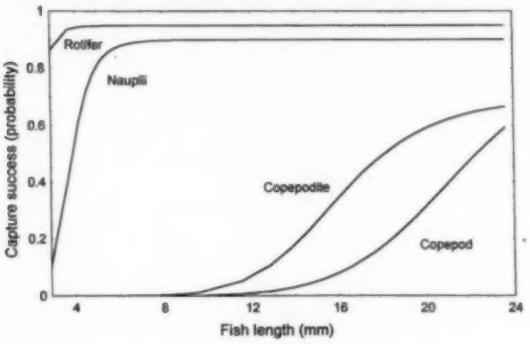
Fig. 2. Capture success as a function of fish length for fish feeding on the different prey in the model.



relationship has also been used in other larval fish IBMs (Rose and Cowan 1993), where

$$(7)\quad RD_{\ell,i} = \frac{PL_i}{2 \cdot \tan\left(\frac{\alpha_\ell}{2}\right)}$$

and

$$(8)\quad \alpha_\ell + 0.0167 \cdot e^{9.14 - 2.4 \cdot \ln(\ell) + 0.229 \cdot (\ln(\ell))^2}$$

Foraging submodel

Fish typically do not attack all prey encountered. Time spent feeding reduces search time, and fish might be expected to chose from encountered prey to maximize benefits (Charnov 1976; Stephens and Krebs 1986). To model this process, we adopted an optimal foraging approach that requires information on capture success and handling time.

Capture success (given attack, CS) was defined as a function of fish size (mass in eq. 9, but written as $CS_{\ell,i}$ for consistency with the other length-based variables) for each of the four prey types (Fig. 2). Because most capture success studies have been conducted with larvae feeding on nauplii (*Artemia* spp. primarily), we chose a capture success function,

$$(9)\quad CS_{\ell,i} = \frac{CSNum_i \cdot mass^2}{CSDen_i + mass^2}$$

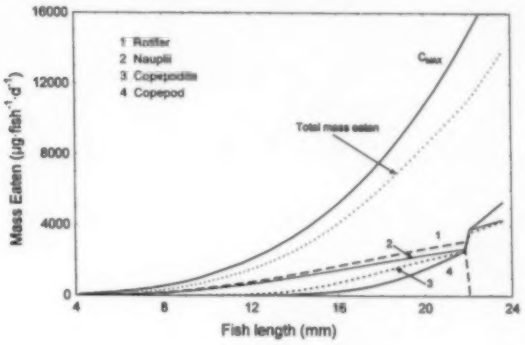
that approximated that from the literature for nauplii and generated a family of curves for the other prey for which capture was less likely for larger prey types for any given fish size (see Fig. 2). Capture success is high (95%) for most fish sizes feeding on rotifers, increases rapidly with larger fish size to a maximum for fish feeding on nauplii, and rises gradually for fish feeding on copepodites and copepods.

We defined handling time (HT, sum of pursuit, attack, and capture times) for each prey type as a function of fish length and prey length using the empirically derived equation of Walton et al. (1992):

$$(10)\quad HT_{\ell,i} = e^{HTInt \cdot 10^{HTSl(PL_i/\ell)}}$$

In this form, handling time decreases rapidly with increases in fish sizes from about 3 to 10 mm in length (depending on prey size) and levels off at 1–2 s/prey for fish > 15 mm in length. Handling times were longer for larger prey types.

Fig. 3. Maximum consumption (C_{max}, equation 13), total mass eaten, and masses of the four prey types (1–4) eaten under nominal conditions (see Table 2). Masses eaten were calculated using optimal foraging methods.



Using these relationships, prey types were ranked according to

$$(11)\quad \frac{Mass_i \cdot CS_{\ell,i}}{HT_{\ell,i}}$$

where $Mass_i$ was the mass of prey type i (µg, Table 1), $CS_{\ell,i}$ was the size-dependent capture success of fish feeding on prey type i (Fig. 2), and $HT_{\ell,i}$ was the handling time for prey type i. Equation 10 indicates that prey types that provide greater mass per unit time required to successfully ingest a single prey received higher ranks.

When the prey were ranked, we assigned profitabilities (benefit–cost ratios) for the ranked prey (j = 1, 2, 3, 4) as

$$(12)\quad \frac{E_{\ell,j}}{T_{\ell,j}} = \frac{\sum_j Mass_j \cdot ER_{\ell,j} \cdot CS_{\ell,j}}{1 + \sum_j ER_{\ell,j} \cdot HT_{\ell,j}}$$

Prey types were included in the diet sequentially on the basis of ranks until the profitability began to decrease (Charnov 1976; Stephens and Krebs 1986). In simulations under nominal conditions, fish < 22 mm in length included all prey types that they could capture in their diet (Fig. 3) and the contribution of larger prey increased as the fish grew. At sizes > 22 mm, the fish in the simulation no longer chose the prey of lowest rank (rotifers) but continued to eat the other prey. These patterns are consistent with empirical observations where prey sizes eaten by larvae increase with fish size (e.g., Hunter 1981; Blaxter 1986; Miller et al. 1990).

Once prey in the diet were determined (k = 1, 2, 3, or 4), the number of each prey actually eaten was calculated via the following series of steps. First, we estimated the number of successful encounters stochastically from encounter rates and capture successes with each prey type (specifically, the realized number eaten was a deviate from a binomial distribution with number of trials equal to the encounter rate per day with probability of success of $CS_{\ell,k}$). This provided an estimate of the number eaten without accounting for time spent searching

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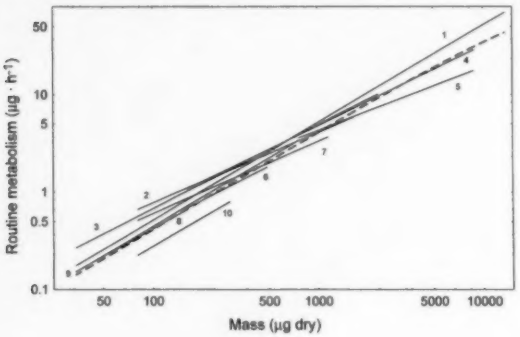
Fig. 4. Routine metabolism from 10 studies corrected to 15°C with a Q_{10} of 3.2. The broken line represents the function used in the model (eq. 17). 1, *Scomber scombrus* (Giguere et al. 1988); 2, *Morone saxatilis* (Eldridge et al. 1982); 3, *Engraulis mordax* (Theilacker 1987); 4, *Clupea harengus* (DeSilva and Tytler 1973); 5, *Pleuronectes platessa* (DeSilva and Tytler 1973); 6, *Clupea harengus* (Almatar 1984); 7, *Pleuronectes platessa* (Almatar 1984); 8, *Achirus lineatus* (Houde and Schekter 1983); 9, *Spartus aurata* (Quartz and Tandler 1982); 10, *Anchoa mitchilli* (Houde and Schekter 1983).



or feeding on other prey. The total amount of time feeding per unit search (1 s) was

$$1 + \sum_k ER_{\ell,k} \cdot HT_{\ell,k}$$

Fish searched for and fed on all prey types during this period. Dividing the number of successful encounters with each prey type per unit search by the total time feeding gave the number of prey per second actually eaten. Multiplying the number eaten per second by the total time feeding per day (ER·Light·60·60·24) and by the mass of each prey yielded the daily mass of each prey type eaten (Fig. 3). Finally, summing over masses of all prey eaten gave the total mass eaten per fish per day (Fig. 3, see also Eggers 1977 for a similar derivation).

If a larva's daily projected consumption exceeded maximum consumption (C_{max}), the mass of food eaten for that day was set to C_{max}, defined as

$$(13) \quad C_{max} = \text{CMaxInt} \cdot W^{\text{CMaxExp}}$$

We obtained parameter values (CMaxInt and CmaxExp, Table 2) by assuming that a 10-µg fish (about 3 mm TL) can eat 200% of its body weight/day and that a 30 000-µg fish (about 23 mm TL) can eat at most 60% of its body weight/day. Larvae at high prey densities can consume from 80 to over 200% body weight/day and C_{max} as a percentage eaten per day often decreases with fish size (Theilacker and Dorsey 1980; Letcher et al. 1996*a*).

Growth submodel

Daily growth (µg/day) was the difference between net input and losses:

$$(14) \quad \text{Growth} = (I \cdot \text{AE}) - \text{TC}$$

Total input (I, µg/day) equaled consumption from the foraging model, and net input was I times assimilation efficiency (AE). Total costs (TC, µg/day) were the sum of metabolism components. Assimilation efficiency, the proportion of ingested food not egested by the fish, was a function of fish mass (W, µg),

$$(15) \quad \text{AE} = \text{AssimMax}(1 - 0.25\, e^{-\text{AssimSh}(W-10)})$$

with parameters AssimMax and AssimSh (Table 2, Buckley and Dillmann 1982). AE ranged from 0.6 to 0.8 and increased rapidly with increases in fish size from 0.6 to the asymptotic maximum (0.8) at about 15 mm. AE values around 0.6–0.7 are typical for first-feeding larvae (Theilacker 1987; Houde 1989; Wieser and Medgyesy 1990). Although AE can decrease with higher ingestion rates (Boehlert and Yoklavich 1984) for juveniles, we did not make AE a function of ingestion rates because little is known about how AE changes with ingestion for larvae and because AE varies with ingestion on an hourly time scale while our feeding time scale was daily.

Total costs (TC) included routine metabolism (RM, µg/day), activity metabolism (ActMetab, µg/day), specific dynamic action (SDA), and egestion (E):

$$(16) \quad \text{TC} = \text{RM} + \text{ActMetab} \cdot \text{Light} + I(\text{SDA} + E)$$

Routine metabolism was defined as a function of fish mass (W):

$$(17) \quad \text{RM} = \frac{\text{MetabNum} \cdot W}{\text{MetabDen} + W}$$

where MetabNum and MetabDen were parameters (Table 2). We derived this relationship by plotting the function through the center of temperature-corrected routine metabolism estimates from 10 studies (Fig. 4). Routine metabolism varies by less than one-half order of magnitude among species. We assumed activity metabolism was 2.5 times routine (range of 1.9 to 2.7 across many species of larvae; Rombough 1988). Activity metabolism was set to zero when the fish were not feeding (nondaylight hours). Both SDA and E were defined as a constant proportion of ingestion (SDA + E = 0.30, see Kiorboe et al. 1987; Wieser and Medgyesy 1990).

Starvation submodel

Two observations motivate the formulation of the starvation submodel. First, Letcher et al. (1996*b*) determined that fish of different sizes die from starvation after losing a constant proportion of their previous maximum body mass (termed the starvation threshold). Second, Kiorboe et al. (1987) and Wieser et al. (1992) showed that the metabolic rates of starving larvae are less than one half (48–71%) those of feeding larvae. To model starvation, we combined a starvation threshold (Thresh) with reduced metabolic rates for fish feeding below maintenance levels. In the model,

$$(18) \quad W_{fin} = \text{Thresh} \cdot W_{max}$$

where Thresh was the proportion of previous maximum body mass (W_{max}) at which a fish starved to death and determined the mass at death from starvation (W_{fin}). Fish reaching W_{fin} for any value of W_{max} died from starvation. W_{max} was reset when the current value was surpassed. Thresholds calculated from the literature range from 0.58 to 0.87 (Table 3); in the model, Thresh was set at 0.75.

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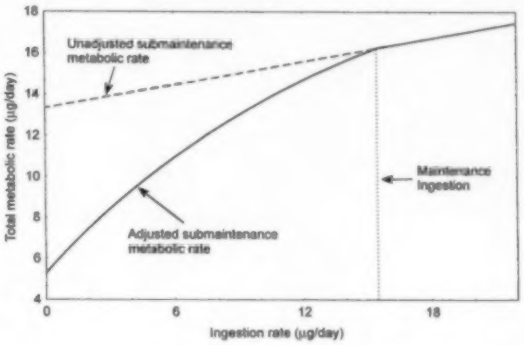
Table 3. Proportional weight loss from the onset of starvation to death by starvation for various species.

Reference	Species	Starvation threshold	Temperature (°C)
Letcher and Bengtson 1993*b*	*Menidia beryllina*	0.87	21
		0.63	25
		0.69	28
McGurk 1984	*Clupea harengus*	0.76	6
		0.79	8
		0.79	10
May 1971	*Leuresthes tenuis*	0.74	18
Rodgers and Westin 1981	*Morone saxatilis*	0.63	21
Toetz 1966	*Lepomis macrochirus*	0.60	24
Werner and Blaxter 1980	*Clupea harengus*	0.59	9
Rice et al. 1987	*Coregonus hoyi*	0.58	12

Note: Starvation threshold is the parameter Thresh in the model and equals mass at starvation divided by the fish's previous maximum mass.

We reduced the total metabolic rate for fish feeding below maintenance such that they would reach W_{fin} after the number of days defined by a size-dependent estimate of the time to starvation for nonfeeding fish (Miller et al. 1988, solid circles in their Fig. 5). The adjusted rate provided intercepts for the size-dependent metabolism-feeding rate relationships (Fig. 5, details in Appendix), which defined metabolic rate between feeding rate = 0 and feeding rate = maintenance. Equation 27 of the Appendix defines submaintenance metabolic rates for all sizes of fish in the model. Metabolic rate increased asymptotically below maintenance and was unaltered above maintenance (Fig. 5).

Fig. 5. Metabolic rate as a function of ingestion rate (solid line) for a 4-mm fish. Below maintenance, metabolic rate was adjusted so that the intercept of the metabolic rate – ingestion rate function caused starving fish to die after the appropriate number of days from a size-dependent time to 50% mortality relation (Miller et al. 1988). Between starving and maintenance, metabolic rate increased nonlinearly, after which it increased as a result of SDA costs only. Both the intercept of the adjusted submaintenance metabolic rate and maintenance ingestion rate will vary with fish size. The unadjusted metabolic rate (broken line) is shown for comparison. Details of the derivation are in Appendix 1.



Predation submodel

We modeled predation on larvae as a series of three independent steps: probability of encounter, attack, and capture. The predator was mobile and gape limited (e.g., many piscivorous fish). To estimate encounter rate, we used the approach of Gerritsen and Strickler (1977), with the modification of Bailey and Batty (1983), which makes the encounter radius of the predator (PredRad, mm) a function of both predator and larval sizes. The encounter radius, combined with swimming speeds of both the predator (v, mm/s) and the larvae (SS, mm/s), and the density of the predators (PredDen, number/mm^3), determined the larvae's encounter rate with predators (ERwPred, number/s). The encounter radius of the predator (PredRad) was a linear function of the predator's swim speed (v, where v = (predator length)PredVSS, see Table 2) and the length of the larvae was

$$(19)\quad \text{PredRad} = \text{PredRadM} + R_L$$

where PredRadM was $0.8v$ (Cowan et al. 1996) and R_L was 2(larval length)/π^2 (Bailey and Batty 1983). The encounter rate was estimated as

$$(20)\quad \text{ERw Pred} = \pi \cdot \text{PredRad}^2 \cdot \frac{\text{SS}^2 \cdot 3 \cdot v^2}{3 \cdot v} \cdot \text{PredDen}$$

Given an encounter, larvae were eaten if they were also attacked and captured. The proportion attacked (PropAtak) nominally equaled 0.5 (arbitrarily chosen to yield mortality rates of about 99%) and we used the summary predator – prey capture success (PPCS) function of Miller et al. (1988, their Fig. 8):

$$(21)\quad \text{PPCS} = 100 - \left(\frac{\frac{\text{Predlen}}{\ell} + \text{CaptNum}}{\text{CaptDen}}\right)^{-\text{CaptExp}}$$

where CaptNum, CaptDen, and CaptExp were parameters (Table 2), PredLen was the predator's length (mm), and ℓ was the larva's length (mm). Because larvae could encounter more than one predator per day, we calculated the daily probability of capture as a set of conditional probabilities. For example, for a larva to be captured on the second encounter, it had first to be encountered twice and avoid capture on the first encounter. Written as conditional probabilities, larval vulnerability (Vuln, day^{-1}) summed over n separate encounters equaled P(encounter = n)·P(not eaten on attempt $n-1$|encounter on attempt n)·P(eaten on attempt n), where $P(x)$ stood for the probability of event x, and $p(x|y)$ was the probability of x given the occurrence of y, i.e.,

$$(22)\quad \text{Vuln} = \sum_n \frac{\lambda_n \cdot e^{-\lambda}}{n!} \cdot (1-z)^{n-1} \cdot z$$

where n indexed encounters, and z was the probability of being eaten once encountered (PropAtak·PPCS). The first term in eq. 22 calculated the random (Poisson) probability of n encounters given an expected number of λ encounters, where λ equaled the mean number of encounters per day (ERwPred multiplied by the number of seconds per day that larvae were vulnerable to predators (Light·24·60·60)). In model runs, n equaled 5

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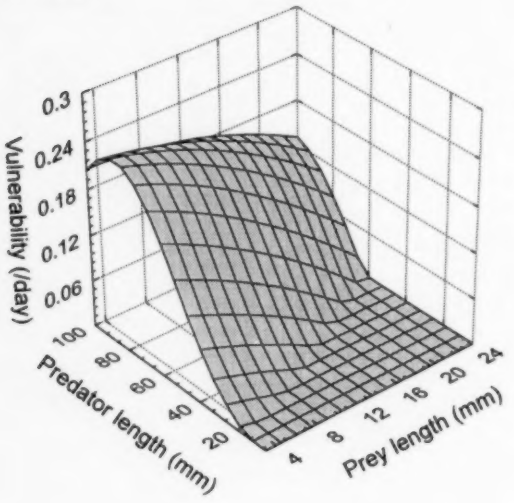
Fig. 6. The larvae's daily probability of being eaten as a function of predator and larval lengths. Vulnerability represents the combined probabilities of encounter, attack, and capture (eq. 22).



because vulnerability always converged to a single value with $n < 5$ (the probability of more than 5 encounters without a successful capture was very unlikely). Larvae that were attacked were eaten if a random number drawn from a uniform distribution (0,1) was less than the probability of being eaten (Vuln).

When larvae were small (4–12 mm), vulnerability increased to a maximum then decreased slightly with increases in predator size (Fig. 6). Vulnerability for larger larvae was lower and increased monotonically with predator size (see Bailey and Houde 1989). The minimum predator size to which larvae were vulnerable also increased with larval size.

Model testing and application

Model evaluation

We used two criteria to evaluate the performance of the model qualitatively. In both cases, we compared model output from a single run under nominal conditions with data reported in the literature. First, we assessed the feeding and growth models by comparing growth efficiency with a published summary (Houde 1989). Second, we compared the time course of starvation and predation mortality with available field estimates (Hewitt et al. 1985). Gross growth efficiency (growth/ingestion) is a useful measure for comparison because it standardizes growth per unit consumption and, except at very low ingestion rates (< 10% body weight/day), declines only slightly with higher ingestion (Checkley 1984; Letcher and Bengtson 1993*b*).

Individual parameter perturbation

To test proportional sensitivity of key model outputs (number of survivors, number eaten, and number starved) to 56 model parameter values (Table 2), we adjusted the parameter values ±10%. Sensitivities were calculated as $(| y_+ - y_- |)/(y_0 \cdot 0.2)$, where y_+ and y_- were the output values with parameters adjusted ±10%, respectively (three replicates) and y_0 was the mean output value (from 10 runs with different random number seeds) using unadjusted parameters. Sensitivities < 1 indicate that the change in the parameter value had a less than proportional effect on the output value, and values > 1 suggest that the parameter had a disproportionately large effect on the response variable. This analysis indicates which parameters have the greatest effect on model results and which functions must therefore be measured with the greatest care during parameter estimation.

Relative effects of intrinsic and extrinsic factors

We conducted a Monte Carlo error analysis (Bartell et al. 1986) to partition the variance in survival into components resulting from variance in intrinsic and extrinsic factors. In a Monte Carlo error analysis, variable values are drawn from a random distribution for each run. Conducting a multiple regression on the output gives partial r^2 values that indicate how much of the variance in the dependent variables can be explained by each independent variable. Partitioning the variance in survival suggests which variables have the largest effects on survival. Error analysis differs from individual parameter perturbation because all variables are modified simultaneously during each run instead of adjusting single parameter values a fixed amount. While this requires many more runs, it provides a more realistic measure of the effects of changing variables because many variable value combinations are possible in a single run. Error analysis also differs from individual parameter perturbation in this case because we adjusted variables (e.g., predator size) and results of calculations (e.g., search volume, total metabolism) instead of parameters (e.g., the slope of a relationship). Adjusting variables indicates the effect of changes in the process itself.

In the error analysis, we adjusted the values of the major variables in each submodel (see Fig. 1) by drawing values from normal distributions with a 10% coefficient of variation (CV, standard deviation (SD)/mean) for each variable. We varied food densities and the predation, starvation, growth, encounter, and foraging submodels (Table 4). We determined actual variable values used in each run by drawing a deviate from a truncated normal distribution (mean = 0, SD = 1, range of –2 to 2) and multiplying nominal variable values (Table 2) by the deviate divided by two times the CV and adding this number to the nominal value. Extending the range of the normal distribution from –2 to 2 and subsequently dividing the deviate by 2 excluded extreme values while expressing most of the normal distribution. For variables with a single value throughout each simulation (predator size, prey density, etc.), the value used was determined at the beginning of each simulation and was maintained throughout the simulation. For variables that were the result of a calculation (total metabolism, search volume, etc.), a single value was also determined for each run but calculations for each fish each day were adjusted with the single deviate. We used a single CV range for all variables because confidence interval estimates were not available for all intrinsic variables. We conducted 1000 runs of the model and used forward stepwise linear regression (SAS Institute Inc. 1989) to partition the variance in survival among the independent variables.

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Table 4. Results from the Monte Carlo error analysis.

Submodel	Variable	Variable type	Survival		Proportion eaten	
			Partial r^2	Predicted change (+10%)	Partial r^2	Predicted change (+10%)
Predation	Predator size	Extrinsic	0.35	−66.96	0.12	0.08
	Predator density	Extrinsic	0.02	−15.69	0.01	0.02
Starvation	Starvation threshold	Intrinsic	0.01	−9.39	0.25	−0.10
Food	Prey density	Extrinsic	0.05	25.80	0.00	0.00
Growth	Total metabolism	Intrinsic	0.15	−40.60	0.26	−0.12
	Assimilation efficiency	Intrinsic	0.15	40.59	0.12	0.07
	Maximum consumption	Intrinsic	0.00	4.28	0.00	0.00
Encounter	Search volume	Intrinsic	0.06	24.94	0.09	0.07
Foraging	Capture success	Intrinsic	0.06	25.21	0.08	0.07
	Handling time	Intrinsic	0.00	−1.20	0.00	0.00

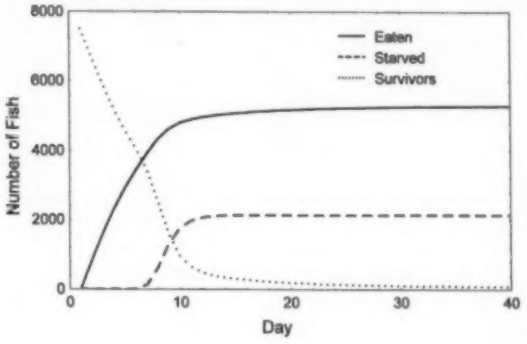
Note: For each run (1000 total), variable values were drawn from a normal distribution using a nominal distribution and a 10% coefficient of variation. The linear effects of key variables from the five submodels were included in the analysis. Partial r^2 values represent the proportion of the variance in survival or the proportion of mortality resulting from predation explained by each variable, and predicted changes indicate the change in number surviving as a result of a +10% change in the actual variable (intercepts; survival, 75.02; proportion eaten, 0.72).

Results

Model evaluation

Gross growth efficiency near maximum consumption (70–90% C_{max}) ranged from 0.21 to 0.29 under nominal conditions in the model. This compares well with Houde's (1989) estimate of 0.29 for 10 species of marine fish larvae. Also, mortality rates in the model were high (> 99%) under nominal conditions, with predation mortality dominating during the first 9 days and a spike of starvation mortality during days 9 and 10 (Fig. 7). After day 11, almost no fish starved, while the predation mortality continued, but at a much lower rate (about 10/day vs. 700/day before day 10). This is similar to the pattern found for larvae of *Trachurus symmetricus*, jack mackerel (Hewitt et al. 1985), where predation dominated mortality but starvation was important briefly during about 5 days after first feeding.

Fig. 7. Cumulative survivorship and mortality as a result of predation or starvation from a single nominal model run.



Individual parameter perturbation

Many parameters had proportional sensitivities greater than 1. Of the 56 parameters tested, 27 had sensitivities > 1 when survival was the response variable, as did 18 and 7 parameters when the output variables were number starved and eaten, respectively. Survival was highly sensitive to changes in many parameters partially because survival under nominal conditions was low (70 individuals or < 1% of starting number). In this case, a change in survival of only 7 fish (0.09% of starting number) in response to a ±10% change in parameter value resulted in a proportional sensitivity > 1. This is a small change, especially when the SD in survival under nominal conditions was 11.3 fish after 10 replicate runs (Table 5) and sensitivities were calculated from three replicate runs. It is remarkable, in fact, that so few parameters (about half of those tested) had proportional sensitivities for survival < 1.

Changes in the length–weight exponent (LWExp) had by far the greatest effect (proportional sensitivity = 63.4) on survival (Table 5). This was due, in part, to the fact that LWExp was an exponent where a 10% change will have a larger effect than if the parameter were a linear part of the function. The intercept of the length–weight relation also had a relatively high proportional sensitivity (4.5, Table 5). Increasing the value of either parameter resulted in lower survival because length was calculated from mass in the model and higher parameter values gave shorter lengths for any given mass.

Changes in the components of predation and growth had particularly large effects on survival. Parameters involved with both encounter (PredVSS, PredRadM) and capture (CaptExp, CaptDen) of larvae by predators had relatively high proportional sensitivities (all > 4.5, Table 5), as did changes in growth (CmaxExp, AssimMax) of the larvae themselves and larval encounter rates with their prey (SSExp). Characteristics of the prey for the larvae (size, density) had relatively small effects, except for the length of rotifers (PyLen1).

While the effects of prey on larval survival were not dominant, they were more important in determining how the larvae died (starvation or predation). The length of rotifers had the second or third highest proportional sensitivity when number of larvae eaten or starved was the response variable (Table 5). This difference in proportional sensitivity between survival and mortality reflects the interaction between predation and starvation mortality. When rotifers were small, 634 more larvae starved and 608 fewer were eaten on average than when they were at the nominal size. The net result was a decrease in survival of 34 fish. Many of the larvae that would have been

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Table 5. Proportional sensitivities (number after parameter name) of the top 10 parameters (out of 56 total) for 3 response variables.

Rank	Survivors		No. eaten		No. starved	
1	LWExp[a]	63.4	LWExp[a]	2.4	LWExp[a]	7.9
2	PredVSS[b]	7.5	Thresh[a]	1.6	PyLen1[c]	3.9
3	CmaxExp[a]	6.7	PyLen1[c]	1.5	Thresh[a]	3.8
4	CaptExp[b]	6.7	MetabNum[a]	1.4	SSExp[a]	3.6
5	AssimMax[a]	5.7	SSExp[a]	1.4	MetabNum[a]	3.5
6	SSExp[a]	5.2	MetabDen[a]	1.3	MetabDen[a]	3.2
7	PredRadM[b]	4.6	LWInt[a]	1.2	LWInt[a]	3.1
8	CaptDen[b]	4.5	PredVSS[b]	1.1	SSInt[a]	2.6
9	LWInt[a]	4.5	SSInt[a]	1.0	AssimMax[a]	2.6
10	PyLen1[c]	4.3	ActMetab[a]	1.0	PredVSS[b]	2.5

Note: Unadjusted (10 runs with nominal parameter values) mean ± SD are as follows: survivors, 70.3 ± 11.3; no. eaten, 5271.8 ± 23.8; no. starved, 2157.9 ± 25.7. See Table 2 for parameter descriptions.

[a]Encounter, foraging, and growth parameters.

[b]Predator-related parameters.

[c]Food-related parameters.

eaten starved because encounter rates with rotifers were lower because of their small size. Like changes in the length of rotifers, changes in the starvation threshold (Thresh) had essentially no effect on survival (proportional sensitivity = 0.36) but also had the second or third highest proportional sensitivity when number of larvae eaten or starved was the response variable (Table 5). While rotifer length and starvation threshold had relatively large impacts on how fish die (predation or starvation), the net result on survival was dampened by the interaction between predation and starvation.

Whether fish were eaten or starved seemed to be influenced mainly by parameters that affected starvation, while overall survival was determined chiefly by those that affected predation. While 4 of the highest 10 sensitivities for survival were predation-related parameters, only one (PredVSS) was in the top 10 for number eaten or starved (Table 5). Besides rotifer length and starvation threshold, number of larvae eaten or starved was most sensitive to changes in parameters related to a larva's encounter rate with food (SSExp, SSInt) and larval growth (MetabNum, MetabDen, AssimMax). Changes in growth were important for both survival and number of larvae starving or eaten because both starvation and predation were functions of size: starvation was the extreme result of slow growth and faster growth reduced vulnerability to predators. Starvation was the dominant source of mortality only under cases of extreme food limitation (and very limited predation pressure).

Error analysis

Overall, the linear (main effects) factors explained 85% of the variance in survival (Table 4); the remaining 15% was due to interactions among the variables. Like the individual parameter perturbation, changes in variables of two of the submodels explained large portions of the variance in survival: the predation submodel (37%) and the growth submodel (30%). The encounter, foraging, and food availability submodels each accounted for only about one sixth as much variance as the predation or growth submodels (each around 5% of the total variance, Table 4). Among particular submodel variables, predator size had the biggest effect on survival, explaining 35% of the variance in survival, while only two other variables could account for >10% of the variance (total metabolism, 15%; assimilation efficiency, 15%). Overall, changes in the extrinsic and intrinsic variables had about equal effects on survival: the extrinsic variables explained 42% of the variance and the intrinsic variables explained 43% of the variance in survival. This result suggests a research focus on characteristics of both the environment (extrinsic factors) and the fish themselves (intrinsic factors) to predict survival.

Regression models from the stepwise analysis (Table 4) indicated magnitudes and directions of effects on survival. Predicted changes gave the effect on number surviving as a result of a ±10% change in each variable. For example, a 10% increase in predator size (50 to 55 mm) would cause a decrease in survival of 67 fish. The magnitudes of the predicted changes mirrored those of the partial r^2 values but the directions were variable. Increasing any of the predation variables or the variables total metabolism, handling time, and starvation threshold had negative effects on survival, while changes in the remaining variables had positive effects.

The results of the Monte Carlo error analyses indicate that the most important processes affecting survival were predation and growth. These results may be somewhat misleading, however, if the natural range of variation in individual variables is very different from 10% CV. For example, if the range of a particular variable is ≪ 10% CV, then the error analysis would likely overestimate its effect. Overestimation was probably not a problem for the dominant extrinsic variable, predator size, which can vary widely depending on year, season, and location (Bailey and Houde 1989).

Effects may have been underestimated if the natural range of a variable is >10% of the mean. This may have been the case for food availability. Prey densities can vary widely spatially and temporally. To assess what level of variability in food availability would be required to cause food availability to have an effect on survival similar to that of variation in the dominant variables (e.g., predator size), we increased the CV in prey densities 2-, 5-, and 10-fold in three separate sets of 1000 runs. CVs for all other variables were nominal (10%), and from these runs, we calculated partial r^2 values as above. Results of this analysis indicated that variation in prey densities about 3 times greater than nominal (i.e., CV = 30%) resulted in an effect on survival equal to that of predator size

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(CVs in prey densities with corresponding partial r^2s: 10%, 0.05; 20%, 0.19; 50%, 0.47; 100%, 0.56). Widely varying prey densities, reflecting differences among years, seasons, or locations, can have a dominant effect on larval fish survival but only when prey density CVs are about 3 times greater than those of the other variables.

While extrinsic and intrinsic factors had equal effects on survival, extrinsic factors had only a small effect on whether fish died from either predation or starvation. Intrinsic factors explained 6 times more of the variation (80 vs. 13%, Table 4) in the proportion of fish that died as a result of predation versus starvation than did extrinsic factors. Total metabolism (26%) and starvation threshold (25%) each accounted for 2 times more of the variance than any other variable, indicating that variation in metabolic rate and sensitivity to starvation is likely to have the largest effect on whether fish die from predation or starvation. Combined with the variance in survival, these results suggest that susceptibility to starvation will generally influence whether fish get eaten or starve but will only have a limited effect on total numbers dying and that predation will have a major impact on survival but only a limited one on whether fish get eaten or starve (explained 12% of the variance, Table 4). Increasing starvation susceptibility reduces the proportion of mortality as a result of predation but has only a limited effect on survival because many of the fish that are starving would otherwise have been eaten. Changes in predation can have a major impact on survival because increased predation pressure through changes in predator size will affect all fish in the cohort, both healthy and unhealthy, and not just the starving fish. This observation highlights the dominant importance of predation as a factor regulating survival.

Discussion

With the model presented in this paper, we asked whether survival in young fish was dominated by a few key processes and whether these processes represented characteristics of the fish (intrinsic factors) or characteristics of the fish's environment (extrinsic factors). The primary result of this exercise was that the extrinsic and intrinsic factors explained similar amounts of variance in survival and that they cannot be viewed in isolation. Predator length and larval growth had the largest effects on survival. Analyzing mortality patterns, Houde (1987) also identified predation and growth rate as important regulators of survival of young fish. In our model, changes in variables relating to these two factors alone explained 67% of the variance in survival, suggesting a focus on predation and growth capacity in future studies.

Growth was the intrinsic variable with by far the largest effect on survival, accounting for 70% of the variance resulting from the intrinsic factors. This result suggests that growth differences among fish, which could be viewed as differences among species (Houde 1987, 1989) or as differences among populations within species (Conover 1990; Present and Conover 1992), can have a significant impact on survival, equaling the effects of the extrinsic variables. On an even finer scale, growth differences among individuals within cohorts can also influence individual survival (Rose and Cowan 1993) as well as cohort survival (Rice et al. 1993). The strong influence of growth on survival suggests that isolating sources of growth variability among individuals, cohorts, populations, and species will contribute to our understanding of survival in young fish and the level of aggregation at which it is determined.

While the genetic basis of growth differences among species is clear, the extent of differences among individuals within a species is less well understood. In our model, we assumed that variability in intrinsic factors has a genetic basis and we identified, by allowing proportional variation in all variables, which factors had the largest effects on survival. In real populations, these critical factors may not all vary proportionately and selection may actually act to reduce variance in the most important variables. If this is true, there may not be sufficient variability in intrinsic factors within a population for individual differences to affect survival and variability in the extrinsic factors could dominate survival. This observation points to a clear need to assess individual variability in intrinsic factors. Once established, the variability could be easily incorporated into an IBM. Care should be taken, however, to assess the covariance structure among variables to avoid unrealistic combinations.

Many of the previous approaches to understanding survival of larval fish have centered on the effects of food density (e.g., Hjort 1914; Cushing 1975; Lasker 1978). In contrast, results from our model showed that changes in food density had only a small effect on survival (explained only 5% of the variance) compared with proportional changes in the other variables. The potential effect of food density may have been underestimated, though, because the range of food densities in the field is probably much greater than simulated here. At very low food densities, survival can be severely limited as most fish starve, but this may be an extreme effect of food density reflecting poor years or unfavorable oceanographic conditions. However, small variations in food density will have relatively little effect on survival. In fact, simulations indicated that proportional variation in food availability would need to be about 3 times greater than variation in predator length for the effects of food availability to equal those of predator length. When prey densities are highly variable (i.e., patchy seasonally or over large spatial scales), differences in prey availability could have a substantial impact on survival.

A criticism of the way in which we varied food densities in the error analysis may be that we adjusted all prey together. In a separate set of 1000 runs, in which we allowed the four prey types to vary independently, the summed effects of the prey explained even less of the total variance in survival (2%). Another possible criticism is that the effects of food density may have been obscured by variability in the intrinsic factors. If the foraging and bioenergetics parameters of a species are sufficiently understood, we could remove this level of variability and test just for the effects of the extrinsic factors. Assuming that this was so, we also varied only the extrinsic factors in an additional set of 1000 runs. The combined effects of the four prey still accounted for only 5% of the variance in survival whereas the changes in predator size and density explained 83%.

The importance of variability in prey densities could also be masked by large predator sizes. To address the question of how small predators would have to be for food availability to have an important effect on survival, we varied predator size and prey densities over a wide range of values (±75% of nominal). Variation in food availability had a negligible effect on

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survival above predator sizes of about 30 mm, but below 30 mm, survival ranged from 0% at low prey densities (< 20% C_{max}) to 20–40% (depending on predator size) when prey densities were high (80% C_{max}). Thus, there was a strong interaction between predator size and food availability such that variation in food availability only had an important effect on survival when predators were smaller than 30 mm.

The effect of the extrinsic factors on survival was dominated by a single variable: variability in predator size alone accounted for 83% of the variance in survival as a result of extrinsic factors. A 10% increase in predator size reduced survival by almost 100% and had a 4-fold greater effect than a 10% increase in predator density and a 2.5-fold larger effect than a 10% increase in prey density. The importance of predation as a factor capable of regulating year-class strength has gained acceptance in the last 20 years (Cushing 1974; Hunter 1981; Sissenwine 1984; Bailey and Houde 1989). The analysis presented here reinforces that notion and suggests that changes in predator size will have a much larger effect than proportional changes in predator density. As with prey density, substantial changes in predator density (e.g., when predators are patchily distributed in space) could have significant impacts on larval fish survival, but relatively small changes in density will not. Predator size may have a larger effect on larval fish survival than predator density because size affects both encounter rates and predator capture success whereas density influences encounter rates only. Research on growth rates and the size structure of predators may yield particularly useful information.

Very high mortality rates are common for fish with pelagic larvae (Sissenwine 1984; Beyer 1989). Disease (Sissenwine 1984) and the indirect effects of physical transport processes (Sinclair 1988; Hare and Cowen 1993) may occasionally influence mortality rates, but most mortality results from predation or starvation (Hunter 1981; Houde 1987). One approach to understanding recruitment has been to divide mortality into components resulting from predation or starvation (Hewitt et al. 1985; Theilacker 1986). However, a focus on whether fish are eaten or starve may yield less information than evaluating characteristics of the survivors (Fritz et al. 1990). This is because predation and starvation interact through compensatory size-dependent processes: starving fish are often more likely to get eaten, thus many fish get eaten that would have starved otherwise. For example, under nominal conditions in model simulations, 82% of the cohort starved without predators compared with 28% with predators, indicating that predators ate 54% of the cohort that would have starved in the absence of predators. Simulations also revealed that the way in which fish died depended predominately on intrinsic factors (characteristics of the fish). Within a species, defined by a particular set of variable values, the relative importance of starvation and predation mortality may be fairly fixed; larvae are either relatively susceptible or unsusceptible to starvation (McGurk 1984; Rice et al. 1987, Table 3). This suggests that examining the relative importance of predation and starvation mortality may be useful mainly for comparisons across species or possibly when predators and food are scarce. Otherwise, results from the simulations suggest that to understand recruitment better we should focus on growth rates of larvae and on the size structure of the predators.

Under most circumstances, predation was the most important source of mortality. This was due in part to the inescapable timing of predation and starvation mortality; larvae can be eaten while feeding endogenously, but they can only starve after they have depleted their yolk reserves. In model runs, about half of the cohort was eaten before any starved. Very high levels of starvation seem likely only under a restricted set of circumstances: minimal predation pressure combined with low food levels. Starvation's minor impact on survival is reinforced by the observation that changes in the starvation threshold, the variable that defined how quickly fish starved, had almost no effect on survival in the individual parameter perturbation or in the error analysis.

While the model presented here was developed to represent a generalized, "typical" planktonic fish larvae, it could be applied to any particular species with planktonic larvae given species-specific parameter estimates. An accurate model of the early life survival for a particular species will be difficult to achieve, however, because the parameters of many functions in the model had a disproportionately large effect on survival. Even if species-specific estimates of all the necessary parameters could be made, the model would still have limited predictive power because model predictions are highly sensitive to variations in many of the parameters. While quantitative predictions may be elusive, predicted qualitative patterns of survival should be robust. To make the best possible model, the following intrinsic parameters should be estimated with the greatest care: the larval length–weight exponent and intercept, maximum consumption exponent, asymptotic assimilation efficiency, the swimming speed exponent, the proportion of the reactive area in which a larva can perceive prey, and parameters in the routine metabolism equation (Table 5).

In a fairly simple model of a young fish's life, we have tried to determine which pieces of a very complicated puzzle might have the biggest impacts on survival. We included components of the fish's biological environment (extrinsic factors) and a detailed description of the fish (intrinsic factors). Within this context, we found that predator size and the fish's capacity for growth had about equally large effects on survival. Both food level and the fish's foraging ability had surprisingly small effects on survival, reflecting the idea that starvation is generally relatively unimportant as a major source of mortality. Our results suggest that small, but proportional, changes in food density will have a relatively small effect on survival compared with those of predator size and larval potential for growth. More extreme variations in food availability, seasonal and large-scale patchiness, or very small predators can increase the importance of food level as a factor controlling larval survival. Further, our results suggest that within a species, good descriptions of metabolic rates and assimilation efficiency will be more important than estimates of foraging ability.

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Appendix

To calculate submaintenance metabolic rates for all fish sizes and feeding rates, we began by defining W_{fin} (the mass at

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starvation) as a function of the proportion of mass remaining after 1 day of starving (β) and the number of days that fish of size W_{max} took to starve (M50):

$$(23)\quad W_{fin} = W_{max} \cdot \beta^{M50}$$

From Miller et al. (1988, their Fig. 5, solid circles), we estimated

$$(24)\quad M50 = M50Sl \cdot \ell$$

where M50Sl was the slope of the relationship between fish length (ℓ) and days to 50% mortality (M50). Because

$$(25)\quad W_{fin} = W_{max} \cdot Thresh = W_{init} \cdot \beta^{M50}$$

where Thresh was the starvation threshold, we could solve for β in terms of Thresh and M50:

$$(26)\quad \beta = Thresh^{\frac{1}{M50}}$$

Percentage weight loss per day for starving fish was represented by 1 – β and weight loss (metabolic rate) in units of mass for starving fish was represented by $(1 - \beta)W$. This estimate is very similar to the relationship in Kiorboe et al. (1987) when their data are adjusted to 15°C with a Q_{10} of 3.2.

We assumed that the metabolic rate below maintenance was a decelerating exponential curve with intercept $(1 - \beta)W$, which rose slowly to maintenance metabolic rate (Fig. 5). We described metabolism below maintenance as a function of ingestion rate (I, μg/day) as follows:

$$(27)\quad SubMaint(I) = (1 - \beta) \cdot W \cdot Maint \cdot \left(1 - e^{-\frac{c \cdot I}{Maint}}\right)$$

where Maint equaled maintenance ration and c was a variable that scaled the slope of SubMaint(I) for the different levels of Maint for fish of different mass. Maint was derived from eq. 14 with growth set equal to 0:

$$(28)\quad Maint = \frac{RM + (ActMetab - 1) \cdot RM \cdot Light}{1 - SDA \cdot AE}$$

and c was derived by setting $y(I) = Maint = I$ and solving eq. 27 for c:

$$(29)\quad c = -\ln\left(\frac{Maint - (1 - \beta) \cdot W}{Maint} - 1\right)$$

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Growth and protein turnover during acclimation to acid and aluminum in juvenile rainbow trout (*Oncorhynchus mykiss*)

Rod W. Wilson, Chris M. Wood, and Dominic F. Houlihan

Abstract: Growth, feeding, and protein synthesis (k_s) and degradation (k_d) in gill, liver, and whole body were measured in juvenile rainbow trout, *Oncorhynchus mykiss*, during 32 days exposure to sublethal acid (pH 5.2) and acid + aluminum (Al) (30 $\mu g \cdot L^{-1}$) in soft water. The only effects observed for exposure to acid alone were depressions of gill k_s (17%) and k_d (27%) after 15 days (data not available after day 15). Exposure to acid + Al caused a loss of appetite, a 73% reduction in whole body growth rate, and a 36% reduction in whole body k_s during the first 7 days; all of these subsequently recovered, although mean body weight was still significantly depressed after 32 days. Gill k_s and k_d were greatly stimulated after 7 days and gill k_s remained elevated after 32 days, suggesting a chronic cost of gill repair and (or) acclimatory processes even after physiological recovery was achieved. However, this elevated cost was small relative to the whole animal protein synthesis budget. Other chronic effects included suppressed liver k_s and k_d, reduced whole body translational efficiency, and enlarged liver size. Conversion of food into growth was paradoxically increased throughout all stages of acid + Al exposure but may have been the result of reduced routine activity.

Résumé : On a mesuré la croissance, la quantité de nourriture absorbée, ainsi que la synthèse (k_s) et la dégradation (k_d) des protéines dans les branchies, le foie et tout l'organisme de jeunes truites arc-en-ciel, *Oncorhynchus mykiss*, au cours d'une exposition d'une durée de 32 jours à une concentration sublétale d'acide (pH 5,2) et d'acide + d'aluminum (Al) (30 $\mu g \cdot L^{-1}$) dans de l'eau douce. La diminution de k_s (17%) et de k_d (27%) après 15 jours (aucune donnée disponible après la 15^e journée) était le seul effet observé dans le cas de l'exposition à l'acide seulement. L'exposition à l'acide + Al entraînait une perte d'appétit, une réduction de 73% du taux de croissance pour tout l'organisme et une réduction de 36% de la valeur de k_s dans tout l'organisme au cours des 7 premiers jours; tous ces paramètres sont redevenus normaux ultérieurement, mais la masse corporelle moyenne était encore assez faible après 32 jours. La k_s et la k_d dans les branchies étaient considérablement stimulées après 7 jours et la k_s dans les branchies était encore élevée après 32 jours, ce qui laisse supposer qu'il y a un coût chronique aux processus de réparation et (ou) d'acclimatation des branchies, même après récupération physiologique. Toutefois, ce coût élevé était faible si l'on tient compte du bilan de synthèse protéique pour tout l'organisme. Parmi les autres effets chroniques, on compte une diminution de la k_s et de la k_d dans le foie, une diminution de l'efficacité de transport dans tout l'organisme et une hypertrophie du foie. La conversion de la nourriture en croissance était paradoxalement accrue à toutes les étapes de l'exposition à l'acide + Al, mais cette observation était peut-être le résultat d'une réduction des activités habituelles.
[Traduit par la Rédaction]

Introduction

Chronic exposure of freshwater fish to sublethal aluminum (Al) concentrations is frequently encountered in low pH soft water owing to the acid-induced leaching of Al from soils and sediments (Wright and Gjessing 1976; Cronan and Schofield 1979; Dickson 1980). It is now clear that at pH values between 4.7 and 5.5, fish kills may be primarily due to the presence of Al rather than the H^+ concentration per se (Schofield and Trojnar 1980; Baker and Schofield 1982). Physiological studies have demonstrated that the gills are the primary target organ of acute Al toxicity and that initial ionoregulatory and respiratory disturbances are associated with surface binding and precipitation of Al on the gills (Playle and Wood 1989, 1991; Reid et al. 1991). However, laboratory studies on prolonged exposure to sublethal Al have shown that acclimation (i.e., increased resistance to lethal Al) occurs with time (usually within 5–17 days; Orr et al. 1986; Wood et al. 1988*a*, 1988*b*; McDonald et al. 1991; Wilson and Wood 1992; Wilson et al. 1994*a*). This may explain the continued presence of fish populations in acidified softwater lakes and rivers containing levels of Al in excess of thresholds suggested by laboratory studies (Wright and Snekvik 1978; Schofield and Trojnar 1980; Kelso et al. 1986).

The acclimation process results from a damage–repair phenomenon involving physiological, biochemical, and structural changes at the gills (McDonald et al. 1991; Mueller et al. 1991; McDonald and Wood 1992). In addition to acclimation,

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R.W. Wilson[1] **and C.M. Wood.** Department of Biology, McMaster University, Hamilton, ON L8S 4K1, Canada.
D.F. Houlihan. Department of Zoology, University of Aberdeen, Aberdeen, U.K.

[1] Author to whom all correspondence should be sent at the following address: Department of Biological Sciences, Hatherly Laboratories, University of Exeter, Prince of Wales Road, Exeter EX4 4PS, U.K. e-mail: r.w.wilson@exeter.ac.uk

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Table 1. Measured water chemistry variables during the chronic exposure regimes.

Exposure group	$[Na^+]$ ($\mu eq \cdot L^{-1}$)	$[Ca^{2+}]$ ($\mu eq \cdot L^{-1}$)	pH	Total [Al] ($\mu g \cdot L^{-1}$)
6.5/0	64.6±3.0 (32)	31.4±1.6 (32)	6.52±0.02 (32)	2.6±0.3 (32)
5.2/0	59.0±3.0 (17)	32.1±1.8 (17)	5.21±0.03 (17)	2.3±0.7 (17)
5.2/Al	58.8±1.9 (32)	31.9±1.8 (31)	5.17±0.03 (32)	29.6±0.5 (31)

Note: Values are given as the mean ± SE, with *n* in parentheses.

some physiological disturbances such as whole body ion content may completely recover with prolonged exposure (Wilson et al. 1994*a*). However, although these gill changes result in increased resistance and reduced physiological disturbances during subsequent Al pulses, certain costs such as reduced growth and chronically impaired aerobic scope and swimming performance are simultaneously incurred (Wilson and Wood 1992; Wilson et al. 1994*b*).

Increased metabolic rate during aerobic swimming in fish acclimated to Al was suggested (though not statistically confirmed) when these fish were compared with controls previously held at circumneutral pH (Wilson et al. 1994*b*). Growth is a more sensitive indicator of subtle increases in energy demand, and impaired growth has indeed been documented for fish exposed to sublethal levels of acid and Al (Sadler and Lynam 1987, 1988; Reader et al. 1988; Mount et al. 1988*a*, 1988*b*; Ingersoll et al. 1990*a*, 1990*b*; Wilson and Wood 1992; Wilson et al. 1994*a*). However, the role of appetite in reduced growth under these stressors has been addressed only rarely (e.g., Wilson et al. 1994*a*), and no studies to date have examined the effects of acid and (or) Al exposure on protein turnover, even though this may provide a sensitive indicator of environmental stresses in general (Houlihan et al. 1994).

The present paper examines some of the potential costs of exposure and acclimation to acid and Al by measuring rates of growth, feeding (appetite), and protein synthesis within the whole body, gills (the key target tissue), and liver (a reference internal tissue). In addition, biochemical correlates of protein turnover (RNA and protein content) have been measured and incorporated with protein synthesis measurements (Garlick et al. 1980) to calculate RNA-translational efficiencies (Millward et al. 1973) in the above tissues. The exposure conditions were created to match as closely as possible our two previous studies (Wilson and Wood 1992; Wilson et al. 1994*a*, 1994*b*). As such, juvenile rainbow trout, *Oncorhynchus mykiss*, were exposed to pH 5.2 in the presence of 30 $\mu g \cdot L^{-1}$ Al for 32 days. All measured variables were compared with two other groups exposed to either pH 5.2 alone, or pH 6.5, both with no Al added. Exposures were carried out in a synthetic soft-water medium relevant to the acid-threatened lakes and rivers of eastern North America and Europe.

Materials and methods

Animal holding and exposure conditions

Juvenile rainbow trout (weight ≈ 6 g) were obtained from Spring Valley Trout Farm and transported to McMaster University, Hamilton, Ontario, where they were initially maintained in continuously flowing dechlorinated tap water ($[Ca^{2+}] \approx 2.0$ meq $\cdot L^{-1}$, $[Na^+] \approx 0.6$ meq $\cdot L^{-1}$, pH ≈ 8) and fed ad libitum. During this holding period fish were divided into three groups of 100 individuals and each given a distinctive freeze brand (Mighell 1969) to allow individuals to be identified during subsequent measurements of growth and protein synthesis.

Following branding the three groups were kept in separate 70-L holding tanks and allowed to recover for 4 days before the transfer to soft water began. Water in the holding tanks was then gradually changed from dechlorinated tap water (11°C) to continuously flowing synthetic soft water (15°C) over 4 days. Flow rates to each tank were approximately 0.6 $L \cdot min^{-1}$ (i.e., 12 volume replacements per day). The synthetic soft-water composition was designed to be similar to that of acid-threatened lakes and streams in eastern North America and Europe (nominal $[Ca^{2+}] \approx 30$ $\mu eq \cdot L^{-1}$, $[Na^+] \approx 60$ $\mu eq \cdot L^{-1}$, $[K^+] \approx 25$ $\mu eq \cdot L^{-1}$, pH 6.5–7.0). A continuous supply of soft water was generated by passing dechlorinated tap water through a reverse osmosis unit (Culligan MP1000), which was occasionally supplemented with product from a deionizing canister (J.W. Anderson Co. Ltd., Dundas, Ont.). The required amounts of reagent-grade $CaCl_2$, NaCl, and KOH solutions were added by peristaltic pump to the main reservoir tank, which supplied all holding tanks, to give the desired ionic composition and pH for the soft-water acclimation period (equivalent to the control group shown in Table 1).

After 8 weeks of acclimation to normal soft water, trout were exposed to one of three conditions: (*i*) normal soft water at pH 6.5 (6.5/0 group), (*ii*) low-pH soft water (nominal pH 5.2; the 5.2/0 group), and (*iii*) low-pH soft water (nominal pH 5.2) with 30 $\mu g \cdot L^{-1}$ Al added (5.2/Al group). To achieve this, the supply to the 5.2/0 and 5.2/Al exposure tanks was acidified in a secondary head tank using an automatic titration assembly (Radiometer TTT80 titrator, PHM84 pH meter, and GK2401C combination electrode). For acidification a 2:1 equivalent mixture of H_2SO_4 and HNO_3 was used to reflect their proportions in the precipitation of the northeastern United States (Galloway and Likens 1981). Vigorous aeration ensured that P_{CO_2} remained at ambient levels (<0.13 kPa). The [Al] was then elevated in the 5.2/Al holding tank by adding an $AlCl_3 \cdot 6H_2O$ stock solution by peristaltic pump to a mixing vessel at the inlet of this tank. The study was conducted from May to August 1992 at 15°C (range ±0.5°C). Water variables (pH, $[Ca^{2+}]$, $[Na^+]$, and total Al) were measured on samples collected daily from each tank (see Table 1).

Feeding regime and measurement of growth

Fish were fed to satiation once each morning using floating trout pellets (Purina Trout Chow). For each exposure tank a separate preweighed bag of pellets was used. At the start of each meal, food was offered in small aliquots (equivalent to less than 5% of the daily ration) once every minute. Feeding was stopped when uneaten pellets still remained after a period of 2 min, and the amount of food eaten per day was calculated from the difference in bag weight at the beginning and end of each day. Feeding rates (percent body weight per day) were calculated from the weight of food eaten and the biomass in each tank. This feeding protocol was adopted 4 weeks prior to, and continued throughout, the experimental exposure period. Organic debris was removed daily from exposure tanks by siphon.

After 8 weeks of soft-water acclimation, fish had reached a body weight of 12.77 ± 0.35 g (mean ± SEM; $n = 300$). To assess subsequent growth rates, all fish were removed at approximately 2-week intervals, individually identified from their brand marks, weighed in a tared vessel containing the appropriate medium, and then returned to their original tank.

Protein synthesis measurement

On days 7, 15, and 32 of the experimental treatments, 10 fish were sampled from each tank to assess rates of protein synthesis from the incorporation of radiolabelled phenylalanine on the basis of the

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method of Garlick et al. (1980). On the above days, feeding was postponed until after the removal of fish for analysis of protein synthesis so that all those used had been starved for approximately 24 h. Fish were individually identified, weighed, and then injected in the caudal vein–artery with a flooding dose of radiolabelled phenylalanine. Successful location of the caudal vein–artery was confirmed by first withdrawing blood into the needle tip. The injection consisted of a 1 mL·100 g^{-1} dose of 150 mmol·L^{-1} phenylalanine (pH 7.5) containing 100 μCi·mL^{-1} (1 Ci = 37 GBq) of L-[2,6-3H]phenylalanine. Following injection fish were transferred to individual darkened tubs containing the appropriate aerated soft-water medium (control, low pH, or low pH + Al). After a 1-h incorporation period, fish were killed by a blow to the head and the whole liver and gills dissected out and weighed. Soft gill tissue was immediately scraped from the cartilaginous filaments and frozen in liquid nitrogen, as were the livers. The remaining carcass was also frozen in liquid nitrogen as quickly as possible. All samples were then stored at –70°C for later analysis.

A 1-h incorporation period was chosen in the present study because we were specifically interested in protein synthesis rates in gill and liver. Preliminary experiments had shown that this was a suitable time for these synthetically active tissues, as specific activities of [^{3}H]phenylalanine within the free pool of these tissues were as predicted for homogeneous distribution. To achieve uniform samples of the whole body, individual frozen carcasses were first ground to a very fine powder in a temperature-controlled mill using a dry ice – methanol mixture as coolant. Whole body and tissue contents of protein and RNA and fractional rates of protein synthesis (k_s) were analysed as detailed in Houlihan et al. (1986). Briefly, tissue samples (approximately 100 mg for gills and livers, 200 mg for powdered whole body) were homogenized in ice-cold 0.2 M perchloric acid (PCA) and the denatured proteins separated by centrifugation. The PCA in the supernatant was precipitated with tripotassium citrate and centrifuged, leaving the free phenylalanine in solution (free pool). Protein in the PCA-extracted tissue pellet was resuspended in 0.3 M NaOH and aliquots were taken for analysis of protein (Lowry et al. 1951) and [^{3}H]phenylalanine content (by liquid scintillation counting). The remaining suspension was acidified with PCA and centrifuged, and the resultant supernatant was analysed for total RNA using the Orcinol method (Munro and Fleck 1966). The pellet, now containing reprecipitated protein and DNA, was washed twice with PCA and then hydrolysed in 6 M HCl for 18 h at 90°C. Following hydrolysis a dry air flow was used to evaporate all traces of the acid and the residues of free amino acids were resuspended in sodium citrate buffer. Phenylalanine in both these protein hydrolysates and the matching free-pool samples was then converted to β-phenylethylamine (using L-tyrosine decarboxylase), extracted using *n*-heptane, and analysed by a ninhydrin reaction. The content of [^{3}H]phenylalanine (now as β-[^{3}H]phenylethylamine) was measured by liquid scintillation counting.

Calculations

Specific growth rates (SGR) for individual fish were calculated from the following equation (Ricker 1979): $SGR = (W_2 - W_1)/((W_1 + W_2)/2) \times 100/t$, where SGR is measured as percentage per day, W_1 is the initial weight (grams), W_2 is the final weight (grams), and t is the length of the growing period (days). Protein growth rates (k_g) of gills and liver were also calculated in the fish sampled for protein synthesis analysis using the same formula (substituting protein content for weight). For these fish, allometric relationships between wet body weight and the whole wet tissue weight were used to estimate initial tissue protein contents for the occasion on which they were weighed 2 weeks previously (corresponding to W_1 in the above equation). The tissue protein contents actually measured at the time of sampling were used as the final values (corresponding to W_2 in the above equation). These allometric relationships were determined from control group fish used during protein synthesis measurement. Additional dissections were carried out on 13 of the remaining fish in the control group at the end of the experiment to increase the range for these allometric relationships (11–83 g). The allometry of the tissues was described using the $\log_e$ transformation of the power relationship $Y = aX^b$ and an exponent of –0.2 (Houlihan et al. 1988), where Y is the wet weight of the tissue (grams) and X is the total wet body weight (grams).

Gross food conversion efficiencies were calculated as described by Brett and Groves (1979), using the dry weight gained (grams) divided by the total weight of dry food consumed for the average fish in each group over a given period (assuming a 73% body water content; Wilson et al. 1994*a*).

The rates of fractional protein synthesis were calculated as $k_s = (SA_b/SA_{fp}) \times (1440/t) \times 100$, where k_s is measured as percentage per day, SA_b and SA_{fp} are the specific activities of phenylalanine (dpm per nanomole) in the protein-bound and free-pool fractions, respectively, and t is the incorporation period (minutes). In practice, true k_s was not measured in all of the tissue samples prepared because of the expense of the assay. However, true k_s was determined in a random selection of liver, gill, and whole body samples ($n = 79$). Estimated k_s values were obtained for these same samples and all other samples from analysis of the [^{3}H]phenylalanine and total protein content of the pellets resuspended in NaOH (see above), assuming a constant phenylalanine content of fish proteins (275 nmol phenylalanine·g $protein^{-1}$). By comparing the true with the estimated k_s in the paired samples, a simple regression relationship was obtained ($r = 0.903$) that was subsequently used to transform all of the estimated k_s to apparent true k_s values. A weight correction exponent of –0.2 was used to convert these individual values of k_s and RNA content to that of a standard-sized 25-g fish (Houlihan et al. 1988). RNA concentrations have been expressed as micrograms of RNA per milligram of tissue protein (capacity for protein synthesis) and the translational efficiency of RNA (k_{RNA}; grams protein synthesized per gram RNA per day) calculated as $k_{RNA} = k_s/[RNA]$ as in Millward et al. (1973).

For liver and gill tissues, protein degradation rates (k_d) were calculated as the difference between k_s and k_g, the specific growth rate for tissue protein.

Acute toxicity tests

On days 7 and 32, 10 fish from each tank were transferred to a single challenge tank supplied with pH 5.2 water containing 200 μg Al·L^{-1}, to assess LT_{50} values (time to 50% mortality) as a measure of changing tolerance to Al. Cumulative mortalities were recorded over the following 96 h or until all fish were dead. LT_{50} values were estimated by log–probit analysis for each group exposed to the challenge. The individual brand marks allowed us to use the same challenge tank for all groups at the same time. Therefore, all groups were exposed to exactly the same challenge conditions.

Analytical techniques

Water samples were analysed for total Al content using an atomic absorption spectrophotometer (Varian AA–1275) with graphite furnace attachment (GTA–95). Atomizer parameters were as described previously (Wilson and Wood 1992). Water [Ca^{2+}] and [Na^+] were analysed on the same machine by flame atomic absorption spectrophotometry.

Statistical analysis

Values are generally reported as the mean ± SEM throughout the text. LT_{50} values were compared between groups using the nomographic methods of Litchfield (1949) and Litchfield and Wilcoxon (1949) at the 5% level of significance. Mean values of all other variables were compared between groups on each day using one-way analysis of variance followed by the Scheffé post hoc test (Scheffé 1953) at the 5% level of significance.

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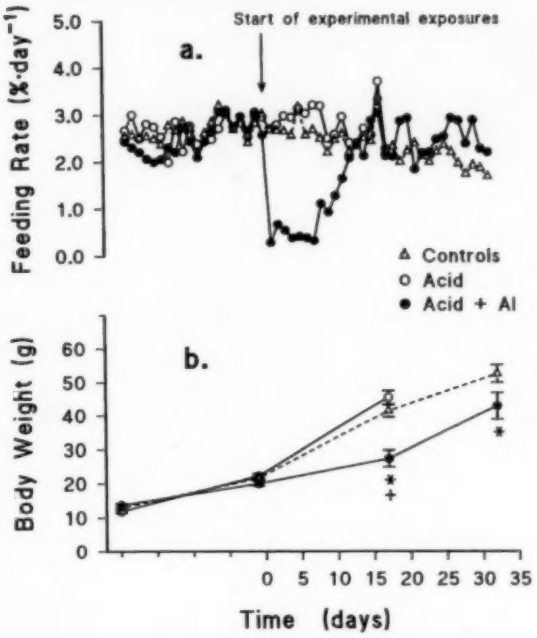
Fig. 1. Group feeding rates (*a*) expressed as a percentage of the average body weight per day in the 6.5/0 group (open triangles, broken line), 5.2/0 group (open circles, solid line), and 5.2/Al group (solid circles, solid line), prior to and during 32 days of the chronic sublethal exposure regimes. (*b*) Mean group wet body weights (±SE, $n = 30–100$) with time during the same chronic sublethal exposure regimes as above. Asterisks indicate significant difference from the 6.5/0 control group, and crosses indicate significant difference from the 5.2/0 group, for the same exposure day.



Results

Mortality and acclimation

No mortality was observed in the control group during the 32-day experiment or in the 5.2/0 group during the first 17 days. Unfortunately, a laboratory mishap caused the loss of all fish in the 5.2/0 tank on day 18. Data are therefore not available for this group following day 17. Exposure to low pH in the presence of 30 $\mu g \cdot L^{-1}$ Al resulted in 30% cumulative mortality over the 32-day experiment.

Increased resistance to lethal Al (200.3 ± 6.9 $\mu g \cdot L^{-1}$ ($n = 6$) at pH 5.24 ± 0.03 ($n = 8$)) was tested on days 7 and 32. As in our previous acclimation studies (Wilson and Wood 1992; Wilson et al. 1994*a*), 5.2/Al trout survived approximately twice as long as the 6.5/0 and 5.2/0 trout during both these acclimation tests (e.g., LT_{50} values on day 7 were 25.2, 12.6, and 15.0 h, respectively). LT_{50} values in the 5.2/0 group were not significantly different from those in the control group on day 7.

Whole tank feeding, growth, and food conversion efficiency

Prior to starting the experiment, the three groups had similar feeding rates of between 2 and 3% body weight·day^{-1} (Fig. 1*a*). From day 0 onwards, feeding rates were similar in the 5.2/0 and 6.5/0 groups with a tendency for daily feeding rates to decline over the course of the study as body weight increased (Fig. 1*b*). Exposure to sublethal Al caused an immediate reduction in feeding rate, reaching a minimum of 0.3%·day^{-1} on day 1. Food intake remained low for the 1st week but recovered during the 2nd week. Thereafter, feeding rates in the 5.2/Al group were comparable with those in the 6.5/0 control group.

Whole tank mean body weights were similar in all three groups (range 19.9–22.2 g) prior to starting the experimental regime (Fig. 1*b*). Net growth was observed in all three groups during the study. However, mean body weight was significantly lower in the 5.2/Al on both days 17 and 32, whereas exposure to acid alone (5.2/0) had no significant effect on body weight when compared with the control group on day 17. Gross food conversion efficiencies were almost identical in the control and acid-exposed fish during the first half of the study (Table 2). However, in the 5.2/Al trout, gross food conversion efficiency was 23% higher than in the other two groups during the first half and 76% greater than in the control group during the second half of the experimental period (Table 2).

Table 2. Calculated gross food conversion efficiencies (%) over the first and second halves of the three exposure regimes, and overall values for the whole study.

Exposure group	Days 0–17	Days 17–32	Days 0–32
6.5/0	32.6	20.4	27.1
5.2/0	32.7	na	na
5.2/Al	40.1	36.0	37.5

Note: Data are not available (na) for the 5.2/0 group after day 17.

Whole body weight-specific growth and protein synthesis rates

SGR and whole body protein synthesis rates were measured in the same 10 fish sampled from each tank on days 7, 15, and 32. Mean SGR in these subsamples of fish mirrored the observed changes in whole tank mean body weights but revealed that the reduced weight in the 5.2/Al group on days 17 and 32 was specifically due to a 73% reduction in SGR during the 1st week of exposure (Fig. 2). During the second and third measurement periods (days 7–15 and 17–32), there were no statistically significant differences in SGR between any of the groups (Fig. 2).

Whole body k_s in the 5.2/Al fish followed the same pattern as SGR, being reduced by 36% on day 7, but was similar to that in the other available groups on days 15 and 32. Table 3 shows that this initial fall in k_s on day 7 was associated with a 25% reduction in whole body RNA content but not translational efficiency (k_{RNA}) or percent protein content. However, on day 32 when SGR and whole body k_s where not significantly different from the values in the control fish, a small but highly significant ($P = 0.005$) depression of k_{RNA} was apparent but without any significant changes in either RNA or percent protein content (Table 3).

Gill protein turnover

None of the variables associated with gill protein turnover was affected by 7 days exposure to acid alone (Fig. 3, Table 4). In contrast to this, and despite a one-third reduction in whole

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Fig. 2. Weight-specific growth rates (SGR; %·day^{-1}) and fractional rates of protein synthesis (k_s; %·day^{-1}) for whole bodies (minus gills and liver) in the three groups (open bars, 6.5/0 group; hatched bars, 5.2/0 group; solid bars, 5.2/Al group) after 7, 15, and 32 days of their respective exposure regimes. Asterisks indicate significant difference from the 6.5/0 control group, and crosses indicate significant difference from the 5.2/0 group, for the same exposure day.

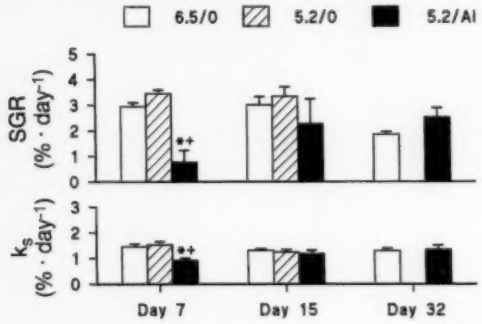
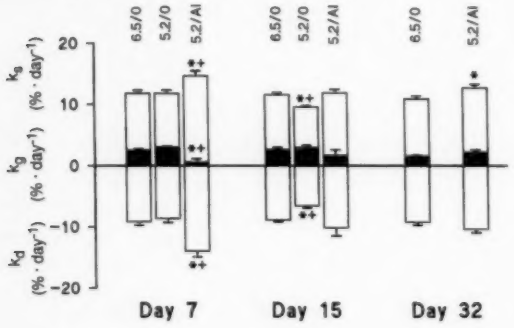


Fig. 3. Fractional rates of protein synthesis (k_s; open bars above the x axis), protein degradation (k_d, calculated from $k_g - k_s$; open bars below the x axis), and net protein growth (k_g; solid bars) in the soft gill tissue of trout after 7, 15, and 32 days of their respective exposure regimes. Asterisks indicate significant difference from the 6.5/0 control group, and crosses indicate significant difference from the 5.2/0 group, for the same exposure day.



body protein synthesis, gill k_s was significantly elevated (by 24%) after 7 days exposure to acid + Al (Fig. 3). An even greater increase in the fractional rate of protein degradation (k_d) was observed in the gills of 5.2/Al trout (up by 53%; Fig.3). These initial changes in gill protein turnover were not attributable to any significant changes in either RNA content or k_{RNA}. The net effect of these changes in k_s and k_d was a reduction in gill protein accretion (k_g; Fig. 3) similar to that observed in the rest of the body (whole body SGR; see Fig. 2), although actual protein content (expressed as mg protein/100 mg wet tissue weight) was elevated slightly relative to that in the 5.2/0 trout (Table 4).

On day 15, both k_s and k_d were significantly suppressed in the gills of trout exposed to acid alone (Fig. 3) and coincided with a 12% reduction in gill RNA content (significantly different from the 5.2/Al group but not the control group; Table 4). In contrast, gill protein turnover in the 5.2/Al trout had returned to a level similar to the controls by day 15.

After 32 days gill k_s was 16% higher in the 5.2/Al trout than in the controls and was associated with an 11% higher gill RNA content (Table 4).

Table 3. Additional parameters related to protein turnover in whole bodies (minus gills and liver).

Exposure time	Exposure group	[RNA] (μg·mg^{-1})	k_{RNA} (g·g·day^{-1})	Protein content (%)
Day7	6.5/0	28.2±1.7	0.51±0.02	11.2±0.5
	5.2/0	29.9±2.3	0.52±0.04	12.0±0.6
	5.2/Al	21.2±1.3*†	0.44±0.02	12.0±0.6
Day 15	6.5/0	24.6±0.8	0.53±0.02	11.3±0.3
	5.2/0	25.9±1.0	0.48±0.04	11.0±0.2
	5.2/Al	23.2±1.8	0.53±0.04	11.4±0.2
Day 32	6.5/0	25.6±2.6	0.52±0.02	11.6±0.8
	5.2/0	na	na	na
	5.2/Al	31.7±3.2	0.43±0.02*	10.9±0.9

Note: Data are not available (na) for the 5.2/0 group after day 17. $n = 10$ except for the 5.2/Al group on day 7 where $n = 9$.

*Significantly different from the 6.5/0 control group, for the same exposure day.

†Significantly different from the 5.2/0 group, for the same exposure day.

Table 4. Additional parameters related to protein turnover in gill tissue.

Exposure time	Exposure group	[RNA] (μg·mg^{-1})	k_{RNA} (g·g·day^{-1})	Protein content (%)
Day 7	6.5/0	81.2±2.4	1.45±0.06	7.08±0.22
	5.2/0	82.0±1.8	1.43±0.06	6.84±0.21
	5.2/Al	91.0±4.5	1.62±0.09	7.92±0.26†
Day 15	6.5/0	90.6±4.1	1.29±0.04	6.63±0.25
	5.2/0	79.7±2.3	1.20±0.03	6.78±0.23
	5.2/Al	94.7±2.2†	1.25±0.06	7.42±0.15
Day 32	6.5/0	78.3±2.6	1.36±0.03	7.37±0.15
	5.2/0	na	na	na
	5.2/Al	87.1±2.4*	1.45±0.04	7.49±0.16

Note: Data are not available (na) for the 5.2/0 group after day 17. $n = 10$ except for the 5.2/Al group on days 7 and 15 where $n = 9$.

*Significantly different from the 6.5/0 control group, for the same exposure day.

†Significantly different from the 5.2/0 group, for the same exposure day.

Liver protein turnover

Net protein accretion rate (k_g) in the livers of 5.2/Al trout was reduced by 74% on day 7 (Fig. 4), paralleling the almost identical reduction in whole body SGR and gill k_g. However, changes in protein turnover in the liver differed markedly from the responses seen in the gills. The only change in liver protein turnover was observed on day 32 when k_s and k_d were reduced by 24 and 35%, respectively, in 5.2/Al trout compared with the controls. No changes in liver RNA content or k_{RNA} were apparent.

Liver protein content (percent wet weight) was increased by 13% following 7 days exposure to acid + Al, despite a reduction in protein growth in this organ, but had returned to

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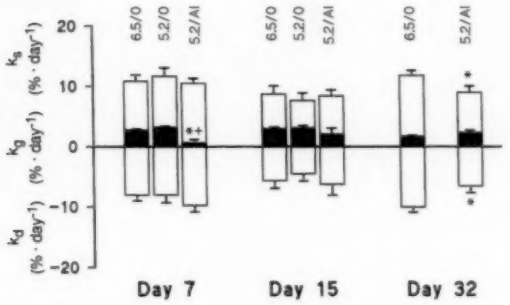
Fig. 4. Fractional rates of protein synthesis (k_s; open bars above the x axis), protein degradation (k_d, calculated from $k_g - k_s$; open bars below the x axis), and net protein growth (k_g; solid bars) in the livers of trout after 7, 15, and 32 days of their respective exposure regimes. Asterisks indicate significant difference from the 6.5/0 control group, and crosses indicate significant difference from the 5.2/0 group, for the same exposure day.



normal by day 15. Despite this recovery of liver protein content, disproportionate growth of whole livers was apparent in 5.2/Al trout by day 32 when the liver somatic index was 31% higher than in the control fish (Table 5).

Table 5. Additional parameters related to protein turnover in liver tissue.

Exposure time	Exposure group	[RNA] (μg·mg^{-1})	k_{RNA} (g·g·day^{-1})	Protein content (%)	Somatic index (%)
Day 7	6.5/0	102.7±3.3	1.07±0.12	10.8±0.2	0.92±0.07
	5.2/0	110.9±2.0	1.06±0.13	10.4±0.2	1.12±0.18
	5.2/Al	101.5±1.8	1.03±0.08	12.2±0.3*†	0.74±0.10
Day 15	6.5/0	102.1±3.4	0.88±0.15	12.9±0.2	1.08±0.04
	5.2/0	105.6±1.5	0.74±0.12	12.7±0.3	1.19±0.04
	5.2/Al	102.9±4.4	0.87±0.13	11.2±0.7	1.21±0.10
Day 32	6.5/0	108.8±2.6	1.08±0.06	12.6±0.2	0.93±0.05
	5.2/0	na	na	na	na
	5.2/Al	105.2±5.1	0.87±0.11	11.7±0.5	1.22±0.09*

Note: Data are not available (na) for the 5.2/0 group after day 17. $n = 10$ except for the 5.2/Al group on day 7 ($n = 9$) and the 6.5/0 group on day 15 ($n = 9$).

*Significantly different from the 6.5/0 control group, for the same exposure day.

†Significantly different from the 5.2/0 group, for the same exposure day.

Discussion

Feeding and growth

We have confirmed that, in trout fed to satiation, a dramatic reduction in appetite is one of the first outward manifestations of exposure to acid + Al. However, for the first time we have additionally shown that the effect this has on growth rate is relatively short lived. After an initial period of 7–12 days, appetite and food intake recover, and thereafter, whole body growth rates return to values not different from trout held at neutral or acidic pH in the absence of Al. It would appear that the chronic reductions in growth reported in previous studies on fish exposed to sublethal acid + Al (e.g., brown trout, *Salmo trutta*, Sadler and Lynam 1987, 1988; Reader et al. 1988; brook trout, *Salvelinus fontinalis*, Mount et al. 1988*a*, 1988*b*; Ingersoll et al. 1990*a*, 1990*b*; rainbow trout, Wilson and Wood 1992; Wilson et al. 1994*a*) may be largely, if not entirely, due to the initial effect that acid + Al exposure has on appetite. However, the recovery of feeding may also be dependent on the dose of Al and (or) the sensitivity of the species, as Mount et al. (1988*b*) observed a prolonged reduction of feeding (up to 193 days) with brook trout exposed to higher Al levels (169 μg monomeric Al·L^{-1}) in pH 4.97 soft water. The effects on feeding and growth are clearly attributable to the presence of Al as they are consistently absent in fish exposed to pH 5.2 alone (see also Wilson and Wood 1992; Wilson et al.1994*a*).

The observation of reduced appetite is not unique to Al exposure. Indeed, a complete cessation of feeding has been reported during the first few days of exposure to sublethal copper in both rainbow trout (Lett et al. 1976) and brook trout (Drummond et al. 1973), and in Atlantic salmon, *Salmo salar*, exposed to sublethal zinc (Farmer et al. 1979), although none of these studies was performed in acidic soft water. A reduction in feeding has also been noted in rainbow trout exposed to lower pH values (4.2–4.7) in the absence of added toxic metals (Brown et al. 1984; Tam et al. 1988). The cause of suppressed appetite in all of these cases is poorly understood but may be related to the increased plasma glucose concentraion often seen during acute and chronic acid or Al stress (Goss and Wood 1988; Playle et al. 1989; Brown et al. 1990; Witters et al. 1990). There is evidence that corticosteroids and catecholamines may be involved in elevated plasma glucose levels, although the relationships among the three are complex (Whitehead and Brown 1989), and some believe that some as yet unidentified factors underlie the hyperglycaemia (Tam et al. 1988). Whatever the cause, Waiwood et al.(1992) argued that hyperglycaemia may reduce appetite by acting as a satiation signal that controls the feeding level, as in mammals. If so, then it would be interesting to see if the subsequent return of appetite between days 7 and 12 is associated with, or preceded by, a similar recovery of plasma glucose concentration.

Despite the initial depression of feeding and growth, fish exposed to acid + Al exhibited a substantially higher gross food conversion efficiency both during the initial period of reduced appetite and after the first 12 days when appetite had completely recovered. The same effect was observed in our previous study on acclimation to sublethal acid + Al (Wilson et al. 1994*a*). This conflicts with the a priori assumption that chronic exposure to metals should act as a loading factor to metabolism and contrasts with the trend for trout exposed to acid + Al to have increased basal oxygen consumption rates (Wilson et al. 1994*b*). The latter were estimated by swimming fish in a respirometer at increasing aerobic speeds (from 20 cm·s^{-1} upwards) and making post hoc extrapolations of oxygen consumption rate back to zero speed. However, neither of these approaches takes into account the behavioural response to acid + Al exposure (see below) and, most importantly, the associated routine metabolic rate of fish under these exposure conditions. To our knowledge, direct measurements of routine metabolic rate under these conditions are not available. However, both in our present and previous studies, trout exposed to acid + Al were noticeably less active than the other two exposure groups. Indeed, reduced spontaneous activity

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has been quantitatively documented in cutthroat trout, *Oncorhynchus clarkii* (Woodward et al. 1989), and brook trout (Cleveland et al. 1986, 1989) exposed to sublethal levels of acid + Al. If reduced activity is accompanied by a lower overall routine metabolic rate, then it could result in a greater percentage of the dietary protein being retained for growth. An improvement in food conversion efficiency might normally be considered an advantage but should be interpreted with caution in the present case. Enhanced food conversion efficiency may occur in the laboratory when the food supply is not limited, but the ultimate effect of a decrease in appetite and routine activity in the wild would surely be a reduction in an animal's overall fitness to feed, avoid predation, and reproduce (Little and Finger 1990).

Whole body protein turnover

Whole body fractional rates of protein degradation could not be estimated in the present study as the k_s values determined by the [^{3}H]phenylalanine technique were smaller than the corresponding k_g values for whole body, which were measured over the preceding 1–2 weeks (Fig. 2). Because the total amount of protein synthesized in the gills and liver only amounted to approximately 15% of the protein synthesized by the remaining carcass, summing the gill, liver, and carcass k_s values did not change this relationship. There are two possible reasons for this discrepancy. Firstly, in fish accurate values for whole body k_s generally require incorporation periods longer than the 1 h that we used (e.g., 2–4 h; Houlihan et al. 1994). This is probably due to the predominance of the white muscle mass in fish, which is relatively poorly perfused (e.g., Wilson and Egginton 1994) and requires longer than other tissues to ensure free-pool swamping with [^{3}H]phenylalanine (Houlihan et al. 1986). Secondly, k_g represents an average protein growth rate over the previous 1–2 weeks during which fish were fed daily, which may be higher than the k_g value at the precise time when k_s was measured (i.e., following 24 h without food). The whole body k_s values quoted in the present paper should be considered relative values to allow comparison between groups rather than absolute estimates.

Changes in whole body protein synthesis rates followed the same pattern as SGR, although the percent drop in whole body k_s after 7 days was only half the equivalent decrease in SGR during the 1st week of acid + Al exposure. Again, this discrepancy may be due to the different times over which these measurements were made (k_s was measured just as the 5.2/Al trout were beginning to recover their food intake whereas k_g is an average value for the preceding 7 days when food intake was at its lowest). Whatever the reason for this discrepancy, the fall in whole body k_s after 7 days acid + Al appeared to be due to a quantitatively similar decline in the number of ribosomal units (RNA content) rather than any changes in the translational efficiency (k_{RNA}). The recovery of whole body k_s once appetite and feeding rate had returned suggests that exposure to acid + Al does not compromise whole body protein synthesis or growth except through the initial effect on appetite and feeding. However, by the end of the 32-day exposure there was a small but highly significant effect of acid + Al on whole body translational efficiency (k_{RNA}) in the 5.2/Al group. Although this suggests that more ribosomes were required to synthesize the same quantity of new protein, the increase in whole body RNA content (23%) was not statistically significant ($P = 0.152$) on day 32.

Because whole body k_s remained similar to controls after the 1st week of acid + Al exposure and translational efficiency was actually reduced, we may tentatively conclude that a reduction in protein degradation rate (although not measured) was involved in the increased efficiency of converting ingested food into weight gain.

Protein turnover in gill tissue

Rates of protein synthesis in gill tissue vary according to feeding state and, hence, whole body k_s and k_g in fish (Houlihan et al. 1988; McMillan and Houlihan 1988; Houlihan 1991). Therefore, analysis of k_s values in gill tissue must take into consideration any difference in feeding rate and (or) whole body k_s between the 5.2/Al trout and the other two exposure groups. For this reason the increase in gill k_s in 5.2/Al trout after 7 days is more than it at first seems, given the substantially depressed whole body k_s at this time. In fact the ratio of gill k_s to whole body k_s in the 5.2/Al trout was almost double that of the control group on day 7. This trend was predicted to some extent as gill damage generally peaks during the 1st week of exposure to acid + Al (e.g., Mueller et al. 1991; Wilson et al. 1994*b*) and is presumably accompanied by an increase in cellular turnover. However, the increases in gill RNA content and k_{RNA} were not statistically significant on day 7, so we cannot specify the underlying cause(s) of this initial increase in gill protein synthesis. Nevertheless, the even greater increase in protein degradation suggests that breakdown of damaged and (or) necrotized gill tissue predominates over the synthesis of new cellular material during this early damage phase of acid + Al exposure. Because both protein synthesis and degradation involve significant energetic cost (Siems et al. 1984; Hawkins 1991) it seems likely that energy consumption by the gills (contrary to that in the whole body) was greatly intensified at this time when damage was still great yet repair and acclimatory processes had been initiated (McDonald and Wood 1992). The intensification of branchial protein turnover while protein metabolism in the remaining whole body is depressed enhances the view that acute Al toxicity is directed principally at the gills.

By day 15, the rate of protein turnover in the gills of 5.2/Al trout was similar to that in the control fish. Time-course studies have shown that, although gill morphology may never completely recover, the majority of changes that do occur (such as mucous and chloride cell hypertrophy) are well established by the end of the 2nd week of exposure to acid + Al (Mueller et al. 1991). The return of gill protein turnover rates in 5.2/Al trout to control levels after 15 days implies that sustaining the new gill morphological status is not particularly costly in terms of protein synthesis. However, this conclusion does not take into account the suppression of gill protein turnover caused by low pH itself (gill k_s and k_d were significantly reduced in fish exposed to acid alone on day 15). On day 15, therefore, rate of gill protein turnover was elevated in the 5.2/Al trout relative to the 5.2/0 group. We also found that gill k_s in 5.2/Al trout was significantly higher than in controls after 32 days of exposure. There is, therefore, some evidence to suggest that continued exposure to acid + Al does produce chronic elevations in protein synthesis within the gills, which implies some increased metabolic cost. This could be associated

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with branchial processes for maintaining increased resistance (acclimation; e.g., increased turnover of mucus), increased cost of combating ionoregulatory problems (e.g., increased production of transport proteins), or simply elevated cell turnover owing to the continuation of Al-induced gill damage. Whatever the reason for this increased protein turnover, the additional metabolic cost will probably be small relative to the fishes' overall energy budget given that the increase in gill k_s only represents about 2.3% of the total protein synthesized by the whole body per day. However, even such a small percent increase could have a significant influence over longer periods of time.

Liver protein turnover

Although protein synthesis in the liver of trout can be influenced by feeding events and ration, McMillan and Houlihan (1988, 1992) showed that after 6 days of either starvation or low ration (0.6% body weight·day^{-1}), liver k_s returned to the same level found in continuously fed trout. Comparison of liver protein synthesis rates between the three exposure groups after 7 days is, unlike the situation in the gills, probably uncomplicated by the lack of feeding in the 5.2/Al group. If this comparison between studies is valid then it would appear that rates of protein synthesis and degradation in the liver are unaffected by 7 days exposure to either acid or acid + Al. Instead, the significant increase in liver protein content of the 5.2/Al trout after 7 days can perhaps be accounted for indirectly by the consumption of liver glycogen during the previous week of near starvation. Glycogen may occupy as much as 7% of the wet weight of trout liver (Waiwood et al. 1992). In addition, glycogen is generally stored with a high water content (Olsson and Saltin 1968). Therefore, a depletion of hepatic glycogen stores would account for most of the increase in liver protein content observed. While a large decline in liver glycogen content has been documented for trout exposed to low pH (Waiwood et al. 1992), this remains to be confirmed for trout exposed to acid + Al.

The liver then does not respond acutely to acid + Al exposure in the same way as the gills. However, continued exposure to acid + Al does eventually lead to a marked suppression of liver protein synthesis and degradation after 32 days. As with the depression of whole body translational efficiency (k_{RNA}) on day 32 this delayed response in the liver may be linked to the very slow accumulation of Al within the internal organs (only detectable after about 10 days; Karlsson-Norrgren et al. 1986; Lee and Harvey 1986; Booth et al. 1988; Witters et al. 1988) compared with the very rapid accumulation of large amounts of Al at the gills (within hours or even minutes; Playle and Wood 1989, 1991; Handy and Eddy 1989). Although the fractional rate of protein synthesis was chronically depressed, the total protein synthesized in the liver per day for a standard-sized 25-g fish (3.3 mg·day^{-1}) was only slightly lower than in the controls (3.5 mg·day^{-1}) because the liver somatic index was substantially higher in the 5.2/Al group on day 32. Therefore, the depressed fractional k_s in the livers of 5.2/Al trout would have had little impact on the overall protein synthetic budget of the whole animal. Liver hypertrophy has also been reported for crucian carp (*Carassius carassius*) exposed to Al for 4 months in an artificially acidified forest pond (Holopainen and Oikari 1992). At present we have no validated explanation for the cause of liver enlargement because there was no statistically significant difference in liver k_g between control and Al-exposed fish on day 32 ($P = 0.083$). However, it is unlikely that changes in either water or glycogen content were involved. Whole body ion status should be fully recovered after about 34 days acid + Al exposure (Wilson et al. 1994*a*), indicating normal fluid distribution, and feeding rates were similar to those in the control fish so we have no reason to invoke different liver glycogen levels.

In summary, we can divide the effects observed in this study into acute (within 7 days) and chronic (after 7 days). The most pertinent acute effect of acid + Al exposure was an almost complete loss of appetite that resulted in depressed whole body protein synthesis during the 1st week. At the same time, gill protein synthesis and degradation were greatly stimulated, an effect we associate with an (assumed) enhancement of gill cell turnover during the early damage phase of acid + Al exposure. Appetite and feeding subsequently recover during continued acid + Al exposure, but some chronic effects persist. These include elevated protein synthesis in the gills, although the cost is small compared with the overall protein synthesis of the animal. Liver protein synthesis and degradation were chronically depressed, as was the whole body translational efficiency. This may indicate a delayed effect of Al on the mechanics of cellular protein synthesis that appears to coincide with the slow accumulation of Al within the internal organs (Lee and Harvey 1986; Karlsson-Norrgren et al. 1986; Booth et al. 1988; Witters et al. 1988; Norrgren et al. 1991). Thus, although a cellular mechanism has not been proposed it is possible that chronic biochemical effects observed within internal tissues are caused directly by elevated tissue Al levels. However, these effects were curiously not observed in the gills where Al levels are probably highest. This could perhaps indicate either a tissue-specific response to Al or perhaps a difference in the nature of Al present within cells, which is dependent on the rate of accumulation. Finally, throughout acid + Al exposure the fish were much more efficient at converting food into whole body growth, a paradox that may be an artifact of our laboratory conditions (feeding to satiation) but that warrants further investigation. We suspect that this may be the result of reduced routine activity and metabolic rate. This behavioural anomaly would be of great consequence to survival and reproduction in the wild where food must be actively sought and predators avoided.

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Mercury speciation and bioaccumulation in lotic primary producers and primary consumers[1]

Walter R. Hill, Arthur J. Stewart, and Guillermo E. Napolitano

Abstract: Mercury speciation and bioaccumulation were strongly affected by trophic position and distance from contamination source in this study of lower trophic levels in two eastern Tennessee streams. Methylmercury and inorganic mercury (Hg(II)) were analyzed in samples of periphyton, grazing fish (stonerollers, *Campostoma anomalum*), and omnivorous fish (striped shiners, *Luxilus chysocephalus*) taken from three sites in an industrially contaminated stream and one site in a reference stream. Inorganic mercury predominated in periphyton and fish samples from all sites, always exceeding 50% of total mercury. However, the proportion of methylmercury increased signficantly with both trophic level and distance downstream. Bioconcentration factors for methylmercury increased 0.5–1.5 log units per trophic level at all sites, implying significant biomagnification of this species. Bioconcentration factors for Hg(II) declined with increasing trophic level at three of the four sites and were 1–2 log units lower than those for methylmercury, providing little evidence of Hg(II) biomagnification. Primary producers and primary consumers are critical intermediaries in the movement and biomagnification of mercury from water to upper trophic levels.

Résumé : Dans cette étude des niveaux trophiques inférieurs dans deux cours d'eau de l'est du Tennessee, la bioaccumulation du mercure et l'espèce bioaccumulée dépendaient étroitement de la position et de la distance trophiques de la source de contamination. On a dosé le méthyl-mercure et le mercure inorganique (Hg(II)) dans des échantillons de périphyton, de poissons brouteurs (roule-caillou, *Campostoma anomalum*) et de poissons omnivores (méné rayé, *Luxilus chysocephalus*) prélevés à trois sites dans un cours d'eau contaminé par des polluants d'origine industrielle et à un site dans un cours d'eau de référence. Le mercure inorganique prédominait dans les échantillons de périphyton et de poissons prélevés à tous les sites, dans lesquels il représentait toujours plus de 50% du mercure total. Toutefois, la proportion de méthylmercure augmentait considérablement avec le niveau trophique et avec la distance vers l'aval. Les facteurs de bioconcentration du méthylmercure augmentaient de 0,5 à 1,5 unités logarithmiques par niveau trophique à tous les sites, ce qui laisse supposer qu'il y a bio-amplification notable par cette espèce. Les facteurs de bioconcentration du Hg(II) diminuaient au fur et à mesure qu'augmentait le niveau trophique à trois des quatre sites, et étaient de 1 à 2 unités logarithmiques plus faibles que ceux du méthylmercure, indiquant ainsi qu'on ne peut guère conclure qu'il y a bio-amplification du Hg(II). Les producteurs primaires et les consommateurs primaires sont les intermédiaires critiques du déplacement et de la bio-amplification du mercure de l'eau vers les niveaux trophiques supérieurs.
[Traduit par la Rédaction]

Introduction

Mercury is a significant environmental contaminant that accumulates to hazardous levels in fish at many locations worldwide. Sources of mercury in aquatic ecosystems include bedrock, effluents from mining and industry, and atmospheric deposition of the by-products of coal combustion and waste incineration. Regardless of source, mercury concentrations are strongly linked to food web structure, with top-level predators generally containing much higher body burdens than lower trophic levels (Cabana et al. 1994). These high concentrations are due almost entirely to the bioaccumulation of methylmercury (e.g., Bloom 1992). Because mercury enters most aquatic ecosystems as inorganic Hg, there is much interest in the process by which inorganic Hg is transformed to methylmercury and concentrated in higher trophic levels.

Relatively little is known about the behavior of mercury in lower trophic levels. Despite their obvious importance as initial steps and intermediaries in food web biomagnification, few studies have examined mercury speciation and bioaccumulation in primary producers and primary consumers. Information about the movement of mercury into benthic food webs of streams is particularly sparse. In a recent study of an uncontaminated lake, Watras and Bloom (1992) reported that the proportions and bioconcentration factors for methylmercury increased dramatically with trophic level within the pelagic food web. The applicability of these results to other aquatic ecosystems (e.g., streams) is unclear.

In this study, we examined mercury speciation and accumulation in lower trophic levels of two small streams. Methylmercury and inorganic mercury were analyzed in periphyton (primary producers), periphyton-consuming stoneroller minnows (herbivores), and striped shiners (omnivores) collected from an industrially contaminated stream and an uncontaminated stream in eastern Tennessee. Our results show that both

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W.R. Hill,[2] A.J. Stewart, and G.E. Napolitano.
Environmental Sciences Division, Oak Ridge National Laboratory, P.O. Box 2008, Oak Ridge, TN 37831–6351, U.S.A.

[1] Publication No. 4453, Environmental Sciences Division, Oak Ridge National Laboratory.

[2] Author to whom all correspondence should be addressed. e-mail: h6t@ornl.gov

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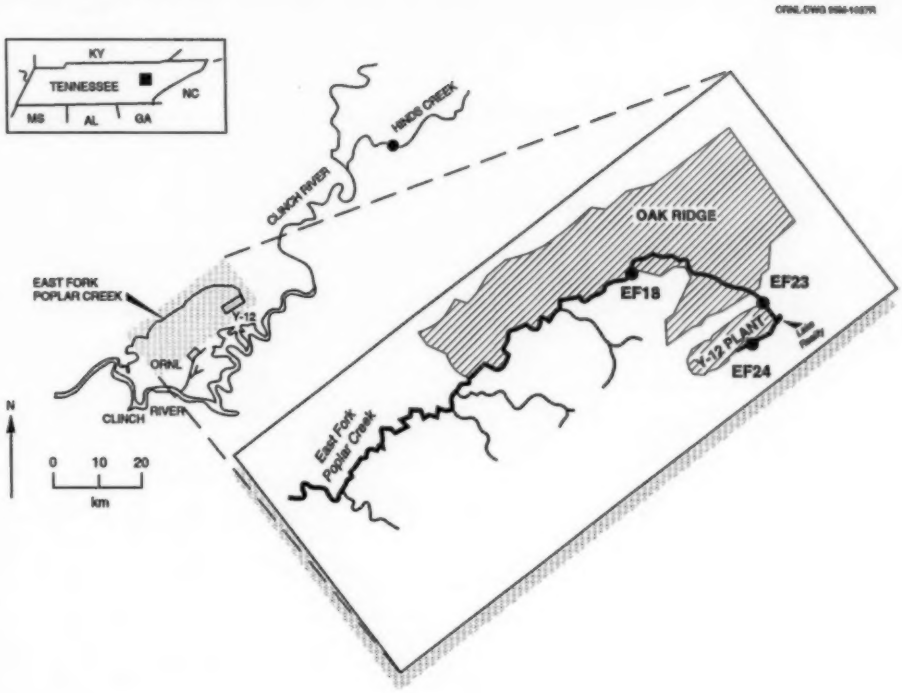
Fig. 1. Sampling locations.



trophic position and distance from the source of contamination strongly influence mercury speciation and bioaccumulation.

Methods

East Fork Poplar Creek (EFPC) is a tributary of the Clinch River that originates in the U.S. Department of Energy's Y-12 Plant in Oak Ridge, Tennessee (Fig. 1). Historical spills of elemental mercury (ca. 250 000 kg) at the Y-12 Plant contaminated sediments and sources of water for EFPC, and despite extensive cleanup activities, mercury concentrations in EFPC fish are still high enough (>1 μg/g) to close the stream to public fishing (Elwood et al. 1987; Southworth et al. 1995). Like most small streams in eastern Tennessee, EFPC contains clear water of moderately high alkalinity and a pH close to 8 (Hinzman 1993). Nutrients from the Y-12 Plant stimulate periphytic primary production in unshaded stretches of EFPC, and in these locations, productive periphyton supports dense populations of grazing stoneroller minnows (*Campostoma anomalum*). Stonerollers are morphologically adapted to harvest periphyton, possessing specialized mouthparts and extended intestinal tracts. Invertebrates are usually only a small proportion of their diet (Becker 1983). Striped shiners (*Luxilus chrysocephalus*) are also abundant in EFPC; these fish are opportunistic predators on invertebrates (especially chironomid larvae) but also consume periphyton and filamentous algae (Becker 1983).

Water, periphyton, and fish were collected in September 1993. Three sites were chosen in EFPC to represent increasing distance from the mercury source in Y-12: (*i*) the upstream site, EF24 (numbers designate distance in kilometres to EFPC's confluence with Poplar Creek), is located closest to the Y-12 mercury source; (*ii*) the middle site, EF23, is located just downstream of Lake Reality (a 1-ha settling basin created in 1989); and (*iii*) the downstream site, EF18, is located in a suburban section of the city of Oak Ridge (Fig. 1). All sites were open (no overhanging trees) riffle-run sections, with depths of 0.5 m or less. Cobbles and gravel were the dominant substrates at each site. Water, periphyton, and fish were also collected at an open site in Hinds Creek, an uncontaminated third-order stream that is located ca. 40 km distant from Oak Ridge. Hinds Creek has served as a reference for estimating background concentrations of mercury, which are usually less than 0.1 μg/g in bluegill and redbreast sunfish from this stream (Southworth et al. 1994).

Two water samples were collected at each site by submerging two 4-L precleaned glass jugs. Care was taken to avoid contaminating the water samples with streambed sediments. Water samples were taken to the laboratory where they were filtered through prerinsed Whatman GFC filters. Filtered water was transferred to 250-mL, acid-rinsed Teflon bottles and shipped on ice at 4°C via overnight courier to the analytical laboratory (Brooks Rand, Seattle, Wash.).

Periphyton samples were collected at each site by brushing the upper surfaces of cobbles that were grazed by stonerollers (stonerollers make distinct grazing scars in periphyton). Three composite samples were made at each site; each sample was composed of periphyton from 5 to 14 individual cobbles. Nylon brushes were used to dislodge the periphyton from the cobbles, and stream water was used to wash the dislodged periphyton into clean stainless steel pans. The resulting slurry was tranferred to clean glass bottles, returned to the laboratory, and centrifuged at 1500 rpm for 1 h. The centrifuged periphyton was then placed in Teflon jars, packed on dry ice, and shipped to the analytical laboratory via overnight courier. Subsamples of the centrifuged periphyton were reserved for dry mass analysis and microscopic examination. The area of each rock brushed was estimated by the foil-gravimetric method (Boston and Hill 1991).

Fish were collected by seining and electroshocking. Stonerollers were obtained at all sites, whereas striped shiners were collected only at EF24 and EF23. Approximately 30 individuals of each species collected at each site were transported live back to the laboratory

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Table 1. Biological data for periphyton and fish samples.

Parameter	EF24	EF23	EF18	Hinds
Periphyton				
AFDM (mg/cm^2)	1.02±0.12	0.81±0.05	1.02±0.02	3.47±0.34
Dry mass (mg/cm^2)	1.40±0.17	1.55±0.13	1.99±0.25	8.20±0.34
Community composition	Chlorophyta, Cyanophyta, diatoms	Chlorophyta, Cyanophyta, diatoms	Chlorophyta, Cyanophyta, diatoms	Cyanophyta, diatoms
Stonerollers				
Length (cm)	10.9±0.3	8.7±0.2	10.0±0.2	10.3±0.2
Mass (g)	13.4±1.1	6.6±0.4	8.6±0.8	8.9±0.6
Shiners				
Length (cm)	9.7±0.2	9.5±0.2	ns	ns
Mass (g)	8.4±0.5	8.9±0.7	ns	ns

Note: Numerical values are means ± 1 SE; $n = 3$ for all periphyton samples and $n = 30$ for all fish samples, except $n = 24$ for EF24 stonerollers. AFDM, ash-free dry mass; ns, not sampled.

where they were anaesthetized (tricaine methanesulfonate), measured (total length), and weighed. The fish were then divided into three composite samples per species and frozen at −100°C. Each sample contained 10 fish, except for one sample of stonerollers from EF24 that included only 4 fish. The composite samples were homogenized with dry ice in a stainless steel blender, and aliquots of the frozen homogenate were shipped on dry ice in Teflon jars to the analytical laboratory via overnight courier.

Total mercury concentrations in water samples were determined by BrCl oxidation, $SnCl_2$ reduction, vapor stripping onto a single gold amalgamation trap, and thermal desorption followed by cold vapor atomic fluorescence spectrometry (CVAFS) detection (Liang and Bloom 1993). Methylmercury concentrations in water samples were prepared by solvent extraction in HCl–KCl and CH_2Cl_2. After back-extracting into pure water, methylmercury was determined by aqueous phase ethylation, room temperature precollection, isothermal gas chromatograph (GC) separation, and CVAFS detection (Liang et al. 1994*a*). Tissue samples (periphyton and fish) were analyzed with the method of Liang et al. (1994*b*), which simultaneously determines inorganic mercury (Hg(II)) and methylmercury. In this method, methanolic KOH extraction and ethylation converts methylmercury and Hg(II) to volatile ethyl derivatives that are precollected onto a trapping column at room temperature and then thermally desorbed into a packed isothermal GC column. Eluted organomercury compounds from the GC column are thermally decomposed into elemental mercury that is detected by CVAFS.

Quality control for the mercury analyses included triplicate analyses of one individual sample from each medium, a matrix spike analysis for dissolved methylmercury, and analysis of standard reference materials. Coefficients of variation for triplicate samples of methylmercury were 6, 1, 4, and 13% for shiners, stonerollers, periphyton, and water, respectively. Coefficients of variation for inorganic mercury were 12, 6, and 9% for shiners, stonerollers, and periphyton, respectively. The coefficient of variation for the triplicate analysis of total mercury in water was 16%. Matrix spike recovery for dissolved methylmercury was 85%. Analysis of two samples of standard reference water (SRM water 1641c from the National Institute of Standards and Technology) resulted in values that were 105 and 93% of the certified value for total mercury. Analysis of two samples of standard reference fish tissue (dogfish muscle DORM-1 from the National Research Council of Canada) resulted in values that were both 98% of the certified value.

Results

Biological data

Periphyton standing crop and community composition were similar at the three EFPC sites. Ash-free dry mass (AFDM) differed little between sites in EFPC, but it was approximately three times greater at the Hinds Creek site (Table 1). Qualitative microscopic examination showed that prostrate chlorophytes contributed the most to community structure at EFPC sites, with filamentous cyanophytes (*Phormidium* and *Calothrix*) and small diatoms making minor contributions. The Hinds Creek community structure was dominated by filamentous cyanophytes (*Calothrix*); small diatoms made minor contributions.

Fish sizes were similar at the four collection sites (Table 1). Qualitative microscopic examination of the gut contents of several stonerollers and shiners collected from EF24 and EF23 confirmed that stonerollers fed almost exclusively on periphyton, and shiners fed on both periphyton and insects (primarily chironomid larvae).

Mercury concentrations

The concentration of dissolved total mercury was an order of magnitude higher at the upstream site in EFPC than it was at the two downstream sites, which did not differ significantly. Lake Reality, the small settling basin between EF24 and EF23, transforms much of the dissolved inorganic mercury in EFPC into particulate-bound inorganic mercury (G. Southworth, Oak Ridge National Laboratory, Oak Ridge, Tenn., personal communication). Dissolved total mercury at the Hinds Creek reference site, 3.3 ± 0.5 ng/L, was an order of magnitude lower than the lowest concentration in EFPC. Dissolved methylmercury in EFPC was three orders of magnitude less than dissolved total mercury and increased rather than decreased with distance from Y-12 (Fig. 2). Dissolved methylmercury was below detection (<0.003 ng/L) in Hinds Creek.

Periphyton in EFPC accumulated large quantities of mercury, most of which was in the inorganic form (Fig. 3). Both Hg(II) and methylmercury in periphyton decreased downstream in EFPC, although the decline in methylmercury was not statistically significant. Periphyton Hg(II) was almost nine times higher at EF24 than at EF18, and it was two to three orders of magnitude lower in Hinds Creek than in EFPC. The concentration of methylmercury in EFPC periphyton was 5 to 10 times that in Hinds Creek periphyton.

Stonerollers and shiners in EFPC contained very high concentrations of Hg(II), exceeding 0.5 μg/g at all sites (Fig. 4). Concentrations in both stonerollers and shiners were highest

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Fig. 2. Dissolved mercury concentrations in filtered stream water. Error bars represent one standard error ($n = 2$). Analysis of variance of $\log_e$ transformed data demonstrated an overall significant site effect for total mercury ($F_{[1,6]} = 493$, $P < 0.001$) and methylmercury ($F_{[1,6]} = 59.3$, $P < 0.001$).

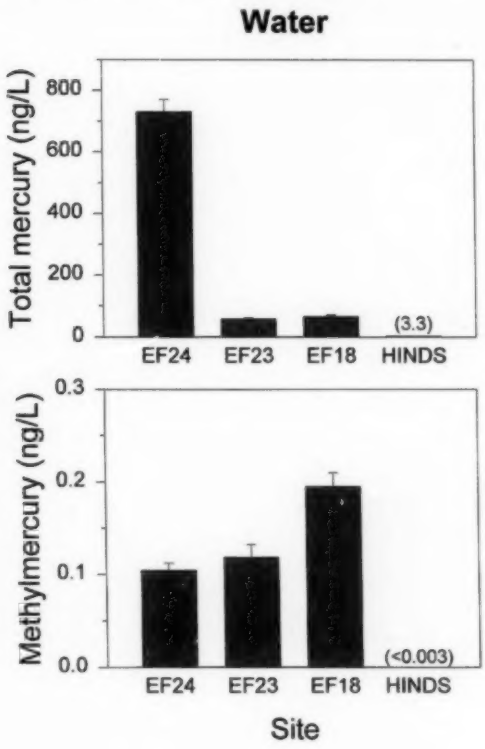
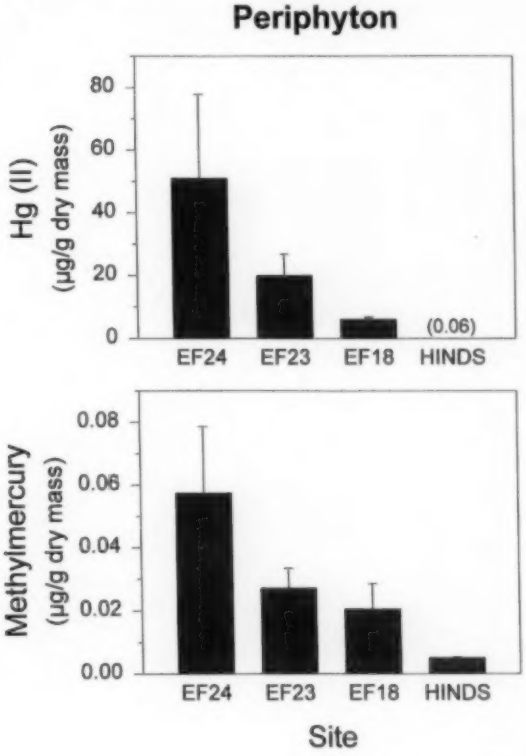


Fig. 3. Mercury concentrations in stream periphyton. Error bars represent one standard error ($n = 3$). Analysis of variance of $\log_e$ transformed data demonstrated an overall significant site effect for total mercury ($F_{[3,8]} = 46.3$, $P < 0.001$) and methylmercury ($F_{[3,8]} = 11.8$, $P < 0.001$).



at EF24 and declined substantially downstream. Stonerollers contained higher concentrations of Hg(II) than did shiners at both of the sites where shiners were sampled. Hinds Creek stonerollers contained low concentrations of Hg(II), one to two orders of magnitude less than those in EFPC. Methylmercury concentrations in EFPC stonerollers and shiners were less than 0.25 µg/g at all sites and were considerably lower than Hg(II) concentrations (Fig. 4). In contrast with Hg(II) concentrations, methylmercury concentrations were actually lowest at EF24. Also in contrast with Hg(II) concentrations, methylmercury concentrations were higher in shiners than in stonerollers. Stonerollers in Hinds Creek contained significantly lower levels of methylmercury than did stonerollers from all sites in EFPC.

The proportion of methylmercury was influenced by both trophic level and proximity to a point source of mercury contamination (Fig. 5). In EFPC, periphyton had the lowest percentages of methylmercury (0.15–0.37%), stonerollers had intermediate values (1.6–21.4%), and shiners had the highest percentages (11.1–40.3%). The trophic effect was also present in Hinds Creek, although methylmercury percentages were much higher for both periphyton and stonerollers than they were in EFPC. The percentage of methylmercury increased downstream in all EFPC samples, but the downstream increase was greater in fish than in periphyton.

Bioconcentration factors

Bioconcentration factors (Bf = log(Cb/Cw), where Cb is the concentration in the biological samples (per unit wet mass) and Cw is the concentration in the water) were calculated for each trophic level. Periphyton concentrations were converted to a wet mass basis by assuming that dry mass was 10% of wet mass (Watras and Bloom 1992). Methylmercury bioconcentration factors consistently increased with trophic level at all sites (Fig. 6). Bioconcentration factors for Hinds Creek in Fig. 6 were estimated by assuming that the concentration of aqueous methylmercury in Hinds Creek was 0.003 ng/L, the minimum detection level. Because the actual concentrations in Hinds Creek were below detection, true bioconcentration factors periphyton and stonerollers are probably higher than represented in Fig. 6. However, the pattern of increasing bioconcentration factor with trophic level still holds true in Hinds Creek, even if the actual concentration of methylmercury is lower than 0.003 ng/L. Bioconcentration factors for Hg(II) were lower than those for methylmercury and generally decreased with increasing trophic level, except at Hinds Creek (Fig. 6).

Discussion

Sorption by primary producers is an important process by

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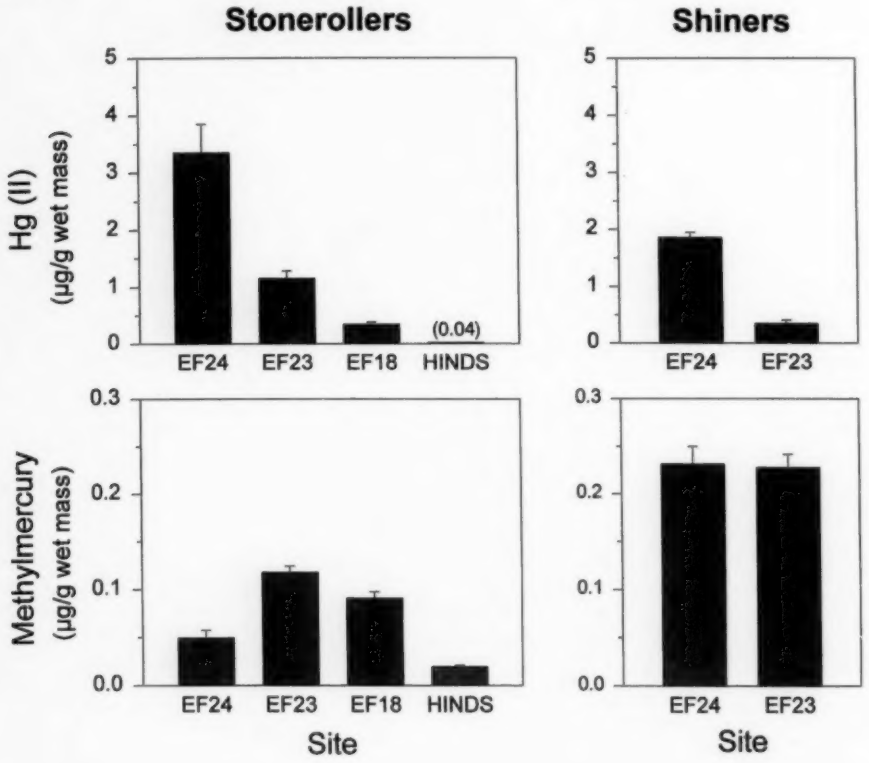
Fig. 4. Mercury concentrations in stream fish. Error bars represent one standard error ($n = 3$). Analysis of variance of $\log_e$ transformed data demonstrated an overall significant site effect for total mercury in stonerollers ($F_{[3,8]} = 184$, $P < 0.001$), methylmercury in stonerollers ($F_{[3,8]} = 54.6$, $P < 0.001$), and total mercury in shiners ($F_{[1,4]} = 155$, $P < 0.001$), but not for methylmercury in shiners ($F_{[1,4]} = 0.22$, $P > 0.50$).



which contaminants such as mercury gain entrance into aquatic food webs. In autotrophic streams such as EFPC and Hinds Creek, periphyton occupies the base of the benthic food web and potentially plays a significant role in the transfer of contaminants to higher trophic levels. In this study, periphyton bioconcentrated both Hg(II) and methylmercury, resulting in concentrations thousands of times greater than those in the water column. Inorganic mercury predominated, especially at EFPC sites, probably as a result of the relatively high concentrations of this form in the water column. Bioconcentration factors for Hg(II) were actually lower than those for methylmercury at three of four sites, suggesting that methylmercury may be sorbed from the water more efficiently than Hg(II). This is consistent with experimental studies of mercury uptake by *Chlorella* (Boudou and Ribeyre 1981) and observational studies on mixed phytoplankton communities (Watras and Bloom 1992). Some methylmercury may be generated within the three-dimensional periphyton matrix by methylation of sorbed Hg(II), particularly if anoxic microzones develop, but the potential contributions of in-place methylation have not been investigated yet.

Mercury within periphyton can be bound to either organic or inorganic substrates. Periphyton naturally includes inorganic sediment particles that settle out of the water column, and although these particles tend to be minor components in clear-water streams where grazers frequently remove sediment from periphyton (e.g., Hill and Knight 1987; Pringle et al. 1993), inorganic sediment particles complexed with Hg(II) (or even methylmercury) undoubtedly contributed at least some of the mercury in our periphyton samples. Dissolved mercury may also sorb to metal oxides that precipitate in periphyton (Newman and McIntosh 1989). Hydrous oxide precipitates of manganese and iron are common on substrates in streams around Oak Ridge (Cerling and Spalding 1982) and may have been incorporated into EFPC and Hinds Creek periphyton. However, mercury also binds strongly to microalgae (e.g., Boudou and Ribeyre 1981; Darnall et al. 1986) and other organic constituents of periphyton (e.g., Hintelmann et al. 1993). The bioconcentration factors for mercury that we found for periphyton were similar to those for phytoplankton (Fisher et al. 1984; Fisher 1985; Watras and Bloom 1992), consistent with the idea that much of the mercury in periphyton is associated with organic substrates. Distinguishing between inorganically bound and organically bound mercury may be important if grazers do not assimilate mercury equally from both substrates, but little is known about this process (see Newman and McIntosh 1989).

Inorganic mercury was also the major form of mercury

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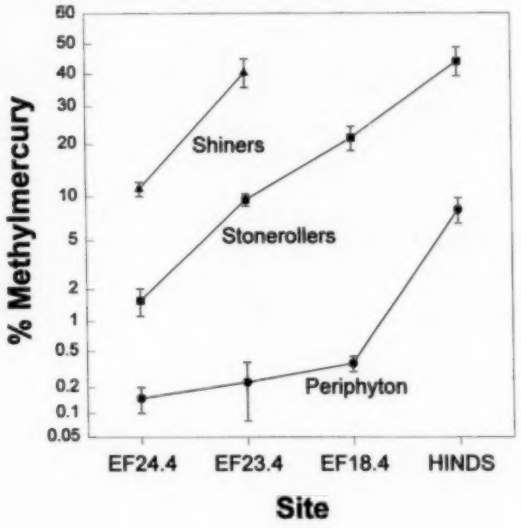
Fig. 5. Methylmercury percentage of total mercury in periphyton, stonerollers, and shiners. Error bars represent one standard error ($n = 3$). Analysis of variance of arcsine transformed data demonstrated an overall significant site effect for periphyton ($F_{[3,8]} = 48$, $P < 0.001$), stonerollers ($F_{[3,8]} = 62$, $P < 0.001$), and shiners ($F_{[1,4]} = 47$, $P < 0.001$).



found in both species of fish. The contribution of the inorganic form to mercury concentrations in EFPC fish varied according to the fish species and the distance from Y-12, but Hg(II) constituted at least 50% of total mercury in all fish sampled in this study, even in uncontaminated Hinds Creek. This predominance of Hg(II) contrasts with almost all previous analyses of mercury speciation in fish tissue, in which methylmercury typically constitutes >85% of total mercury (e.g., Huckabee et al. 1974, 1979; Bloom 1992). The high percentage of Hg(II) in our samples is in part due to the analysis of whole bodies rather than fillets. Whole body samples include visceral tissue, which is the principal site of Hg(II) accumulation, whereas fillet samples are almost all muscle tissue, which accumulates primarily methylmercury (Boudou and Ribeyre 1985; Allen 1994). Muscle tissue has been the focus of most mercury analyses of fish because humans consume fish muscle. Despite greater concentrations of Hg(II) in viscera, however, most samples of whole bodies should still be 80–90% methylmercury (Bloom 1992; Watras and Bloom 1992).

Some of the elevated levels of Hg(II) in EFPC fish may have been due to direct absorption of Hg(II) from the water column because dissolved concentrations of Hg(II) were so high. However, even if one assumes that dissolved Hg(II) concentrations are as high as 1 μg/L and that direct uptake causes a 1000-fold bioconcentration (Newman and Doubet 1989; Southworth et al. 1995), Hg(II) concentrations in fish tissue should be only 1 μg/g, a value considerably lower than the maximum concentration found in EFPC stonerollers. Other fish in EFPC that do not consume periphyton (redbreast sunfish) contain only 15% Hg(II), even though these fish are also exposed to high concentrations of dissolved Hg(II) (Southworth et al. 1995). In addition, Hg(II) concentrations in Hinds Creek were very low (3.3 ng/L), yet Hg(II) constituted >50% of total mercury in stonerollers from this stream.

We suggest that diet is largely responsible for the high proportion of Hg(II) in stonerollers and shiners. Both species consume periphyton, which contains very high concentrations and proportions of Hg(II). Shiner diets also include significant numbers of small invertebrates (e.g., chironomids), which probably have higher levels of methylmercury than periphyton, and so the higher proportion of methylmercury in shiners (compared with stonerollers) is consistent with their somewhat higher trophic position. Differences between stonerollers and shiners in Hg(II) content cannot be explained by larger quantities of periphyton in the long intestinal tract of stonerollers at the time of sampling: gut contents constitute ca. 2% of total stoneroller mass (W.R. Hill, unpublished data), so even though Hg(II) is five times more concentrated in periphyton than in the fish as a whole, gut contents account for less than 5% of total Hg(II) in stonerollers.

The low proportion of methylmercury in periphyton and its consumers is similar to the low proportions found in the few analyses of lower trophic levels from other aquatic ecosystems. Phytoplankton and zooplankton from an uncontaminated Wisconsin lake contained only 13 and 29% methylmercury, respectively (Watras and Bloom 1992), filter- and deposit-feeding marine mussels contained less than 50% methylmercury (Riisgaard et al. 1985), and freshwater benthic invertebrates in a number of locations contained 50% or less methylmercury (Hildebrand et al. 1976; Huckabee et al. 1979). The high proportions of methylmercury found in predatory fish are likely to occur through the concentrating effect of sequential enrichment of methylmercury at each step in the trophic chain leading to top predators (Watras and Bloom 1992).

Mercury speciation in all trophic levels was strongly influenced by sampling location. The proportion of methylmercury in stonerollers and shiners increased significantly downstream in EFPC, and although the increasing methylmercury trend in EFPC periphyton was not statistically significant, the proportion of methylmercury in Hinds Creek periphyton was many times higher than that in EFPC. We believe that the rise in methylmercury proportions is related to increasing distance from anthropogenic sources of Hg(II), which appears to be methylated by in-stream processes. Dissolved Hg(II) may be methylated as a result of the cumulative effect of anaerobic bacterial activity in stream sediments or periphyton, or it may occur in the water column itself. Downstream increases in water column methylmercury within the Wabigoon/English River–Lake system were reported by Parks et al. (1989), who concluded that the water column was an important site for methylation. The higher proportion of methylmercury in periphyton and stonerollers from Hinds Creek may be related to its non point source of mercury, which is presumed to be a combination of atmospheric and geological.

Bioconcentration factors indicated distinct differences in the accumulation of methylmercury and Hg(II). First, bioconcentration factors for methylmercury were generally greater than those for Hg(II), especially for stonerollers and shiners, whose methylmercury bioconcentration factors were 2–3 log units higher than their Hg(II) bioconcentration factors. Second, methylmercury bioconcentration factors increased with

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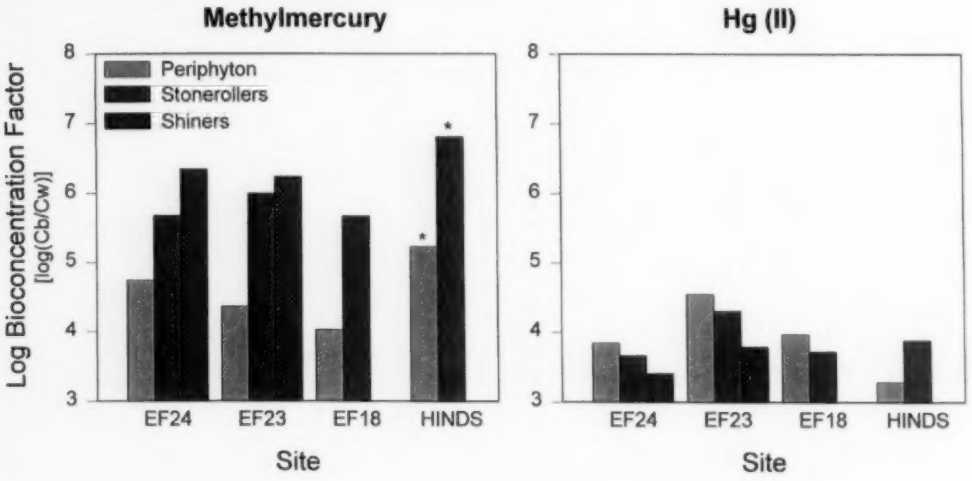
Fig. 6. Bioconcentration factors (log_{10} transformed) for methylmercury and Hg(II) at each stream sampling site. Asterisks indicate that Hinds Creek methylmercury bioconcentration factors are minimum values calculated by using the detection limit for dissolved methylmercury (0.003 ng/L).



increasing trophic level at all sites, rising by approximately 0.5–1.5 log units per trophic level. Bioconcentration factors for Hg(II) increased with trophic level only in Hinds Creek, and the increase was much smaller than the increase in methylmercury bioconcentration factor. These results agree with experimental evidence that trophic transfer of methylmercury is much more efficient than that of Hg(II) (e.g., Boudou and Ribeyre 1981). The results also resemble those of Watras and Bloom (1992), who found that bioconcentration factors for methylmercury increased 0.5 log units per trophic level in a pelagic food web. Increasing bioconcentration factors suggest that each successive trophic level acquires methylmercury from a progressively more concentrated source (Watras and Bloom 1992). These findings are consistent with the observed increase in the proportion of methylmercury in higher trophic levels.

Some caution should be exercised in comparing contaminant levels in different media collected in a single sampling event. Contaminants accumulate in biota over time, so sampling on one occasion may not adequately characterize average exposure levels. Mercury concentrations in water can change rapidly in response to hydrological conditions, and consequent changes in the mercury concentrations in periphyton and fish may lag considerably behind those in the water. At this time, it is unclear how quickly equilibrium can be established between mercury in water, mercury in periphyton, and mercury in grazing fish. The data we present here should be considered a snapshot of potentially dynamic conditions, subject to both physicochemical and biological variability.

This study is the first to examine mercury speciation and bioaccumulation in both primary producers and primary consumers in lotic food webs. The results suggest that patterns of mercury accumulation depend greatly on the specific form of mercury examined and that simple measurements of total mercury obscure the dynamics of mercury behavior in lower trophic levels. Because of the apparent importance of lower trophic levels in biomagnifying mercury, more research on primary producers and primary consumers is needed to increase knowledge of the processes by which mercury and other contaminants reach unsafe levels in fish.

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Beverton and Holt life history invariants result from optimal trade-off of reproduction and survival

A.L. Jensen

Abstract: The Beverton and Holt invariants are three special relations among life history parameters: $Mx_m = C_1$, $M/K = C_2$, and $L(x_m)/L_{inf} = C_3$, where M is the instantaneous natural mortality rate per individual, x_m is age at maturity, K is the von Bertalanffy growth coefficient, $L(x_m)$ is length at maturity, L_{inf} is asymptotic length, and C_1, C_2, and C_3 are constants. In this study the Beverton and Holt invariants were obtained from maximization of the fecundity function that optimizes the trade-off between survival and fecundity; this approach applies conventional fishery models and provides estimates of the constants C_1, C_2, and C_3. These estimates can be compared with the estimates obtained by regression of $1/M$ on x_m, M on K, and $L(x_m)$ on L_{inf} across species and across populations within species. The values of the constants obtained from regression across species agree closely with the theoretical values of the constants. In fishery assessments, natural mortality is often approximated using growth parameters, and the analyses reported here indicate that the relations are not just statistical relations, but rather result from fundamental ecological relations among the parameters.

Résumé : Les invariants de Beverton et Holt sont trois relations spéciales entre des paramètres du cycle de vie : $Mx_m = C_1$, $M/K = C_2$ et $L(x_m)/L_{inf} = C_3$, dans lesquelles M est la valeur instantanée du taux de mortalité naturelle par individu, x_m est l'âge à maturité, K est le coefficient de croissance de von Bertalanffy, $L(x_m)$ est la taille à maturité, L_{inf} est la taille asymptotique et C_1, C_2 et C_3 sont des constantes. Dans cette étude, on a obtenu les invariants de Beverton et Holt en maximisant la fonction de fécondité qui optimise le compromis entre la survie et la fécondité; cette approche met en application des modèles classiques de pêche et fournit des valeurs estimatives pour les constantes C_1, C_2 et C_3. On peut comparer ces valeurs aux valeurs estimatives obtenues par régression de $1/M$ sur x_m, M sur K et $L(x_m)$ sur L_{inf} pour les espèces et pour les populations à l'intérieur d'une espèce. Les valeurs des constantes obtenues par régression pour les espèces concordent étroitement avec les valeurs théoriques de ces constantes. Lors d'évaluations dans le domaine des pêches, on établit souvent une approximation de la mortalité naturelle à partir des paramètres de croissance; de plus, les analyses signalées dans cette étude indiquent que les relations ne sont pas uniquement des relations statistiques, mais sont plutôt le résultat de relations écologiques fondamentales entre les paramètres.
[Traduit par la Rédaction]

Introduction

Life history parameters have been estimated for many fish populations, and close relations among parameters have been found across species and across populations within species (e.g., Jensen 1981, 1985; Beverton and Holt 1959; Beverton 1992; Roff 1984; Pauly 1980; Charnov 1993). Some of these relations are of practical value as they provide a simple and economical method for preliminary estimation of natural mortality (e.g., Pauly 1980). Three special relations that Charnov (1993) called the Beverton and Holt invariants are $Mx_m = C_1$, $M/K = C_2$, and $L(x_m)/L_{inf} = C_3$, where M is the instantaneous natural mortality rate per individual, x_m is age at maturity, K is the von Bertalanffy growth coefficient, $L(x_m)$ is length at maturity, L_{inf} is asymptotic length, and C_1, C_2, and C_3 are constants. These invariant relations have been discussed at length by Beverton (1992), Pauly (1980), and Charnov (1993). Charnov (1993) showed that maximization of the net reproductive rate with respect to age at maturity, which is equivalent to maximization of evolutionary fitness, gives $Mx_m = C_1$, and the other Beverton and Holt invariant relations then can be obtained, but with additional assumptions (Charnov 1993). In this study, I show that the Beverton and Holt invariants also can be obtained from ecological theory, and that the relations result from a trade-off between survival and fecundity. Furthermore, the ecological approach applies conventional fishery models and gives estimates of the constants C_1, C_2, and C_3. I thank Ray Beverton for suggesting that I look at these relations and Erik Charnov for indicating that eq. 7 could be obtained from Roff's equation (eq. 5) using my equation for the inflection point.

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A.L. Jenson.[1] School of Natural Resources and Environment, University of Michigan, S.T. Dana Building, 430 East University Street, Ann Arbor, MI 48109–1115, U.S.A.

[1] Author to whom all correspondence should be addressed.

Derivation of the Beverton and Holt invariants

Let the fecundity function $f(x) = l(x)m(x)$ be the number of female young produced by females of age x, where $l(x)$ is the proportion of females that live to age x and $m(x)$ is the number of females produced by a female of age x. With indeterminant growth, $f(x)$ will increase to a maximum and then decrease as a result of the decrease in $l(x)$ and increase in $m(x)$ with age. To maximize reproductive output, age at maturity (x_m) should

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Table 1. Summaries of statistical models fitted to Pauly's (1980) data on von Bertalanffy growth parameters (K), asymptotic weights (W_{inf}), water temperatures (T), and natural mortalities (M).

Dependent variable	Independent variables	Regression coefficients	R^2	SE
Log M	Log K	0.51, 0.95	0.65	0.62
Log M	Log W_{inf}	0.88, –0.22	0.34	0.85
Log M	Log T	–2.73, 0.82	0.21	0.94
Log M	Log K, log W_{inf}	0.67, 0.87, –0.04	0.66	0.62
Log M	Log K, log T	–0.53, 0.87, 0.36	0.69	0.59
Log M	Log T, log W_{inf}	–1.32, 0.84, –0.22	0.56	0.70
Log M	Log K, log T, log W_{inf}	–0.49, 0.68, 0.46, –0.08	0.71	0.57
M	K	0.21, 1.45	0.77	0.56
M	K	0, 1.60	0.74	0.58

Note: R^2 is the adjusted coefficient of determination and SE is the standard error of the estimate. The number of observations was 175, the mean of M was 0.945, the mean of log M was 0.559, and the total sum of squares was 213.99. All models except the last one have an intercept estimated from the data, and the intercept is the first coefficient listed for each model.

occur when $f(x)$ is maximal, for if x_m occurred before the maximum, then energy for growth would be diverted into reproduction before reproductive efficiency peaked, and if x_m occurred after the maximum, mortality would claim individuals at their reproductive peak before they reproduced (Roff 1984).

Roff (1984) applied the function $f(x)$ to obtain the optimal age at maturity. Fecundity was assumed proportional to weight, $m(x) = aW(x)$, and weight was described by the von Bertalanffy equation

$$(1) \quad W(x) = W_{inf}(1 - e^{-Kx})^3$$

where W_{inf} is asymptotic weight and K is a growth parameter. Mortality after hatching was described as

$$(2) \quad dN/dx = -M(x)N$$

where $M(x)$, the instantaneous mortality rate per individual, is a function of age. The proportion of young that survive to the age of maturity is

$$(3) \quad l(x_m) = \exp(-\overline{M}x_m)$$

where $\overline{M}$ is the first moment of the function $M(x)$ between 0 and x_m. Substitution of eqs. 1 and 3 and the solution of eq. 2 into the fecundity function give

$$(4) \quad f(x) = \exp(-\overline{M}x_m)\exp(-M(x - x_m))haW_{inf}(1 - e^{-Kx})^3$$

where h is the number of eggs per unit weight of female and M is the instantaneous natural mortality rate per individual for adults. Taking the derivative of eq. 4 with respect to x, setting the result to zero, and simplifying gives (Roff 1984)

$$(5) \quad x_m = \frac{1}{K}\log\left(\frac{3K + M}{M}\right)$$

Jensen (1985) showed that age at maturity across different populations was closely related to age at the inflection in the von Bertalanffy equation for growth in weight. Suppose that age of maturity does occur at the inflection point, then

$$(6) \quad x_m = \log(3)/K$$

and substitution of eq. 6 into eq. 5 and simplifying gives

$$(7) \quad Mx_m = 1.65$$

Equation 7 is the first Beverton and Holt invariant relation; it actually was proposed by Charnov and Berrigan (1990). Substitution of eq. 7 into eq. 6 gives the second Beverton and Holt invariant:

$$(8) \quad M/K = 1.50$$

The third Beverton and Holt invariant was obtained from the von Bertalanffy equation for growth in length:

$$(9) \quad L(x) = L_{inf}(1 - e^{-Kx})$$

Substitution of eq. 6 into eq. 9 and division by L_{inf} gives

$$(10) \quad L(x_m)/L_{inf} = 1 - \exp(-\log(3)) = 0.66$$

which is the third Beverton and Holt invariant.

Discussion

Obtaining the Beverton and Holt invariant relations by maximizing the fecundity function provides estimates of the constants C_1, C_2, and C_3, which can be compared with the values obtained when parameters across species are regressed on one another. From regressions of $1/M$ on x_m Charnov and Berrigan (1990) reported that for fish in general Mx_m is approximately 2 as compared with the 1.65 obtained here; Beverton (1963) reported that Mx_m was 1.54 for cod, 1.54 for flatfish, and 3.33 for brown trout. Applying the data for K and M of Pauly (1980) for 175 different species gives $M/K = 1.60$ as compared with the 1.50 obtained here. For the third Beverton and Holt invariant, $L(x_m)/L_{inf}$, Beverton and Holt (1959) and Charnov (1993) reported values of 0.4 to 0.8, which nicely enclose the predicted value of 0.66. The theoretical values of C_1, C_2, and C_3 are close to those obtained by regression of $1/M$ on x_m, Z on K, and $L(x_m)$ on L_{inf} across species.

Pauly (1980) suggested application of the equation

$$(11) \quad \log M = -0.2107 - 0.0824 \log W_{inf} + 0.6757 \log K + 0.4627 \log T$$

to predict natural mortality from easily estimated growth parameters. Equation 11 was obtained by Pauly (1980) from data on asymptotic weights W_{inf} (grams), growth coefficients K (year^{-1}), instantaneous natural mortality coefficients M (year^{-1}), and temperature (degrees Celcius) for 175 different species. Equation 8 and the theoretical analysis indicate that the relation between M and K is not just a statistical relation, but rather that it results from fundamental ecological relations among life history parameters. To determine if K alone was adequate for prediction of M, as indicated by eq. 8, all possible regressions with dependent variable log M and independent variables log K, log W_{inf}, and log T were fitted to Pauly's (1980) data. The model with dependent variable M and independent variable K also was fitted. In terms of either the standard error or proportion of variation in M predicted, the simple linear regression of M on K is as good as the multiple linear regression model (Table 1).

The Beverton and Holt invariants are fundamental ecological relations that do not depend on the functional form of the growth equation. Many equations have been applied to describe indeterminant growth; the von Bertalanffy growth equa-

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Table 2. Parameters of parabolic equation fitted to growth data.

	a	b
Atlantic salmon	331.47	0.634
Brook trout	47.29	0.952
Lake trout	90.96	0.766
Lake herring	117.34	0.531
Lake whitefish	360.61	0.227
Rainbow smelt	152.42	0.315
Muskellunge	429.78	0.409
Cutthroat trout	126.42	0.754
Rainbow trout	28.34	1.328
Gizzard shad	282.77	0.231
Bloater chub	82.96	0.561
Rock bass	64.82	0.605
Green sunfish	32.71	0.764
Bluegill sunfish	82.54	0.453
Largemouth bass	190.32	0.404
Yellow perch	9.901	1.364
Walleye	336.653	0.287
Pacific herring	26.08	0.141
Flatfish	104.93	0.607

Note: All data are from Scott and Crossman (1973) except the Pacific herring and flatfish data, which are from Gulland (1969).

tion is the most widely applied model (e.g., Ricker 1975, 1979), but it has been criticized because it has an asymptote, which implies that there is an upper limit to growth, and no term has units of length per unit time as might seem reasonable for a growth model (Knight 1968; Ricker 1975, 1979). Beverton and Holt (1957) used the von Bertalanffy equation because it couples nicely with the exponential mortality equation to give a differential equation for yield that can be solved in closed form. Perhaps the simplest equation applied to describe indeterminant growth is the parabolic equation

$$(12) \quad L(x) = ax^b$$

where $L(x)$ is length at age x, and a and b are parameters (Ricker 1979). Equation 12 has neither an asymptote nor an inflection point. With eq. 12, the fecundity function $f(x)$ can be written as

$$(13) \quad f(x) = \exp(-Mx_m) \exp(-M(x - x_m)\ h(ax^b)^3$$

and taking the derivative of eq. 13, setting the result to zero, and simplifying gives

$$(14) \quad Mx_m = 3b$$

Equation 14 is again the relation observed when $1/M$ is regressed against x_m across different species or across separate populations of the same species. The other Beverton and Holt invariant relations in terms of growth equation 12 are $M/K = 2.73b$ and $L(x_m)/L_{inf} = 0.66$. The parameter b can be estimated from growth data and the parameters C_1 and C_2 can be calculated and compared with the values obtained with the von Bertalanffy equation and with regression.

I obtained growth data for 19 species using readily available resources and fitted the parabolic equation using nonlinear least squares (Table 2). The mean value of b was 0.5964. Substitution of the estimated value of b into eq. 14 gives $Mx_m = 1.79$ and substitution of b into eq. 7 gives $M/K = 1.63$. These calculated values of C_1 and C_2 are again close to those obtained by regression of $1/M$ on x_m and M on K across species and also are close to the values obtained with the von Bertalanffy equation.

The evolutionary fitness maximization through optimization of the net reproductive rate R_o gives the same results (Charnov 1993) as the ecological optimal trade-off between survival and mortality, but maximization of R_o does not provide estimates of the parameters. It is of interest that species evolve such that age at maturity occurs when the fecundity function is a maximum, and comparative and simulation studies indicate that if mortality of a fish population increases through harvesting, the age at maturity decreases to at least partially offset the increased mortality (Jensen 1985, 1991).

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Genetic evidence for the persistence and divergence of native and introduced sockeye salmon (*Oncorhynchus nerka*) within Lake Washington, Washington

Andrew P. Hendry, Thomas P. Quinn, and Fred M. Utter

Abstract: The genetic population structure of Lake Washington sockeye salmon (*Oncorhynchus nerka*) was investigated using the analysis of variation in allelic frequencies at protein-coding loci. Population subdivision was considerable (average F_{ST} using the four highly polymorphic loci was 0.057) and two divergent population groups were identified (separated by an average genetic distance of 0.014). One population group (Cedar River, Issaquah Creek, and a Lake Washington beach) showed a genetic affinity to collections of sockeye salmon from Baker Lake, Washington. In contrast, the other population group (Bear Creek and Cottage Creek) was distinct from either of the putative non-native ancestral populations (Cultus Lake, B.C., and Baker Lake). We inferred that the former group was comprised of fish of the Baker Lake lineage (transplanted to Lake Washington in the 1930s and 1940s) and that the latter population group was predominantly of native ancestry. Cultus Lake fish were not closely related to any of the other populations but there was some evidence for introgression among the different ancestral lineages within Lake Washington. Allelic frequency differences among several populations of a common origin provided evidence for two possible types of genetic divergence: between ancestral and derived groups and among different derived populations.

Résumé : On a étudié la structure génétique de la population de saumons sockeye (*Oncorhynchus nerka*) du lac Washington en examinant la variation de fréquences alléliques aux locus assurant le codage des protéines. Il y avait subdivision considérable de la population (la valeur moyenne de F_{ST} établie avec quatre locus hautement polymorphes était de 0,057), et on a identifié deux groupes de population divergents (séparés par une distance génétique moyenne de 0,014). Un groupe de population (rivière Cedar, ruisseau Issaquah et une plage du lac Washington) présentait une affinité génétique vis-à-vis des collections de saumons sockeye du lac Baker, Washington. Par contraste, l'autre groupe de population (ruisseau Bear et ruisseau Cottage) était distinct des deux populations ancestrales présumées non indigènes (lac Cultus, en Colombie-Britannique, et lac Baker). Nous avons déduit que le premier groupe de population était constitué de poissons de la lignée du lac Baker (transplantés dans le lac Washington dans les années 1930 et 1940) et que le dernier groupe était surtout d'origine ancestrale indigène. Les poissons du lac Cultus n'étaient pas étroitement apparentés à ceux des autres populations, mais certaines données semblaient indiquer qu'il y avait eu introgression parmi les différentes lignées ancestrales du lac Washington. Les différences de fréquences alléliques chez plusieurs populations d'origine commune venaient appuyer deux types possibles de divergence génétique, soit entre des groupes ancestraux et des groupes dérivés et entre des populations dérivées.
[Traduit par la Rédaction]

Introduction

Unlimited migration among conspecific populations (discrete breeding aggregations) can homogenize genetic variation and lead to apparent panmixia (Wright 1978; Allendorf and Phelps 1981). Natal homing, a phenomenon exhibited by Pacific salmon (*Oncorhynchus* sp.) and many other organisms, can reproductively isolate different populations, limiting gene flow among them (Labelle 1992; Quinn 1993; Tallman and Healey 1994). This restriction on gametic exchange allows the genetic divergence of populations through random processes (genetic drift and mutation) and natural selection (Ehrlich and Raven 1969; Endler 1986; Slatkin 1987; Altukhov and Salmenkova 1991). Although undisturbed populations may eventually approach an equilibrium between genetic drift, selection, and migration (Wright 1969; Slatkin 1994), introduced fish can disrupt this balance (Hindar et al. 1991; Krueger and May 1991).

An interaction between introduced (non-native) and indigenous (native) conspecifics can lead to (*i*) success of the introduced fish through replacement of the native fish, (*ii*) persistence of the native fish coupled with failure of the introduced fish, (*iii*) introgression of the native and introduced fish, or (*iv*) coexistence of both native and introduced fish as reproductively isolated populations that breed at different times and (or) in different places. If both native and non-native fish contribute to a population mixture, the relative genetic influence of each ancestral group may vary among the derived populations,

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A.P. Hendry,[1] T.P. Quinn, and F.M. Utter. University of Washington, School of Fisheries, Box 357980, Seattle, WA 98195-7980, U.S.A.

[1] Author to whom all correspondence should be addressed. e-mail: hendry@fish.washington.edu

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reflecting some combination of stocking intensity and variation in adaptive life history traits. Pacific salmon provide an opportunity to investigate the outcome of such genetic interactions because many introductions have been made into locations that already harbored conspecifics.

Outside their native range, the transplantation of salmonids has been extensive, establishing a number of new populations (Withler 1982; Krueger and May 1991; Harache 1992). Within their native range, however, only two introductions provide unequivocal evidence for the establishment of self-perpetuating, anadromous salmon populations (Withler 1982). In Frazer Lake, Alaska, sockeye salmon (*Oncorhynchus nerka*) became self-sustaining after intensive stocking and the removal of a barrier to anadromous migration (Blackett 1979). In Lake Washington, Washington, sockeye salmon transplants also became self-perpetuating within a modified drainage (see below), which nonetheless lacked blockages to migration both before and after the introductions (Ajwani 1956). Thus, the success of sockeye salmon introduced to Lake Washington represents a rare and possibly unique example, within the native range of the species, of a Pacific salmon transplant that established a self-perpetuating anadromous population in a system without a historical barrier to anadromy (Withler 1982).

The historical distribution and abundance of *O. nerka* within Lake Washington is poorly understood, owing to a lack of detailed records. Nevertheless, substantial numbers of kokanee (the non-anadromous form) and limited runs of sockeye salmon (the anadromous form) were probably present at the turn of the century (Seale 1895; Evermann and Meek 1898; Rathbun 1900; Cobb 1911). In 1917, the Cedar River (formerly a tributary to the Duwamish River) was diverted into Lake Washington and a ship canal was constructed that connected the lake more directly to Puget Sound (Ajwani 1956). Associated changes in the drainage pattern and lake level (a drop of 3 m) were generally assumed to have resulted in the extinction or severe depletion of anadromous sockeye salmon within the watershed (Ajwani 1956; Woodey 1966). Nevertheless, there is some genetic evidence that native populations may have persisted in certain areas (Seeb and Wishard 1977).

The first recorded introduction of non-native sockeye salmon into Lake Washington occurred in 1917, but the exact source of these fish is unknown and they apparently did not establish any substantial populations (Woodey 1966). No other introductions occurred until the late 1930s, when an intensive stocking program began (Royal and Seymour 1940). Juvenile sockeye salmon from Baker Lake in the Skagit River system, Washington, were stocked into Bear Creek (576 000 in 1937), Issaquah Creek (1 553 000 from 1937 to 1944), and Cedar River (969 000 from 1937 to 1945). Returns of these fish to Issaquah Creek were then used for hatchery supplementation in Cedar River until 1945 and in Issaquah Creek until 1963 (Woodey 1966). The only other external source of sockeye salmon planted into Lake Washington was Cultus Lake in the Fraser River system, British Columbia, from which fingerlings were planted into North Creek (24 000 in 1944) and Issaquah Creek (60 000 from 1950 to 1954). For detailed reviews of sockeye salmon introductions into the Lake Washington drainage, see Ajwani (1956), Woodey (1966), and Hendry (1995). Currently, anadromous sockeye salmon and non-anadromous kokanee spawn throughout the Lake Washington watershed but are only abundant in certain areas (Ron Egan, Washington Department of Fish and Wildlife, 600 Capitol Way North, Mail Stop 43151, Olympia, WA 98501–1091, U.S.A., unpublished data).

The present study investigated the genetic population structure of Lake Washington sockeye salmon using the analysis of variation in allelic frequencies at protein-coding loci. This approach has frequently been used to investigate ancestral relationships and to evaluate population differentiation in Pacific salmon (Allendorf and Phelps 1981; Gharrett and Thomason 1987; Utter 1991). For sockeye salmon, biochemical genetic analysis has succeeded in discriminating broad geographical population groups (e.g., Withler 1985; Foote et al. 1989; Wood et al. 1994), populations from nearby lakes and streams (e.g., Wilmot and Burger 1985; Quinn et al. 1987; Grant et al. 1980; Varnavskaya et al. 1994*a*), and populations from different spawning locations within lake systems (e.g., Altukhov and Salmenkova 1991; Varnavskaya et al. 1994*b*). For Lake Washington, ancestral origins of the different sockeye salmon populations were inferred through comparisons with the fish currently found in Baker and Cultus lakes. Distinctions noted within and among native and introduced groups were then evaluated for evidence of genetically distinct populations within the Lake Washington drainage. The results were considered with respect to the interaction of native and introduced conspecifics and the dynamics of population divergence after an introduction.

Methods

Collections

In the fall of 1992 and 1993, adult sockeye salmon were collected from each of five spawning areas within the Lake Washington drainage (Cedar River, Issaquah Creek, Bear Creek, Cottage Creek, and the Pleasure Point beach; Fig. 1*b*). The numbers of fish collected from each location varied in proportion to the size of the spawning population. Additional collections included adult sockeye salmon from Baker Lake in 1992 and 1993 and juvenile sockeye salmon from Cultus Lake in 1992 (the latter were provided by Chris Wood, Department of Fisheries and Oceans, Pacific Biological Station, Nanaimo, BC V9R 5K6, Canada). Kokanee were collected from Issaquah Creek in 1993 (13 adults) and from Bear and Cottage creeks in 1992 (3 adults). Few kokanee were collected because the spawning populations in both years were extremely limited.

Tissue samples (liver, heart, eye, and skeletal muscle) were obtained from all adult and juvenile fish and stored at −80°C. These samples were then processed using horizontal starch gel electrophoresis and stained for specific enzyme activity reflecting distinct allozyme loci and alleles (Aebersold et al. 1987). Twelve enzyme systems coded by 20 loci (Table 1) were screened in a minimum of 40 fish from each sockeye salmon collection. Two other loci (*LDH-B2** and *TPI-1.2**) were screened in a minimum of 40 individuals from each of the 1993 sockeye salmon collections. All of the kokanee were screened for all 22 loci. Samples were run in several tissues and using several buffer systems to ensure accurate scoring. Banding patterns were interpreted using established protocols (Utter et al. 1987) and standardized gene nomenclature (Shaklee et al. 1990).

Allelic frequencies were determined by direct count for all loci except *PGM-1** and *LDH-A1**. For these two loci, heterozygotes could not be readily distinguished and so allelic frequencies were estimated from the square root of the frequency of null homozygotes (assuming Hardy–Weinberg equilibrium). Loci were categorized as high polymorphic ($q > 0.05$), low polymorphic ($0.05 > q > 0$), or

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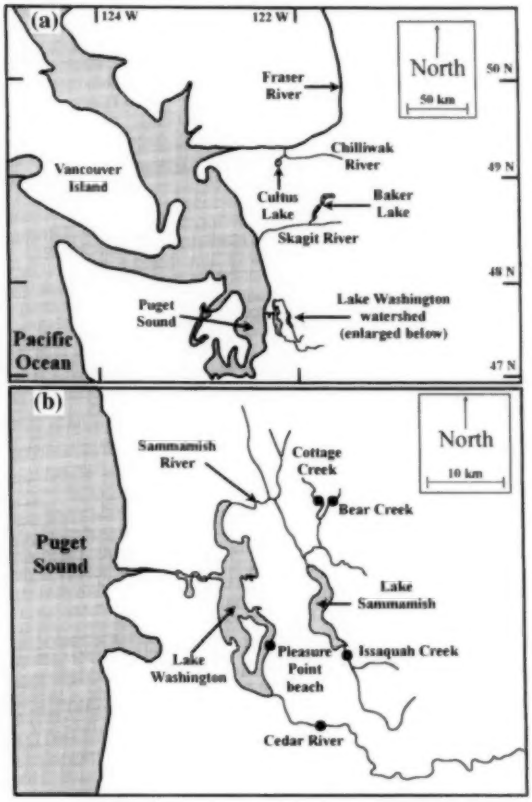
Fig. 1. Locations of the different collection areas. (*a*) The relative locations of Cultus Lake, Baker Lake, and Lake Washington. (*b*) Collection locations within the Lake Washington watershed, designated with a ●.



monomorphic ($q = 0$), where q was the frequency of variant alleles in one or more collections.

In each collection (population- and year-specific), genotype frequencies were tested for deviations from those expected under Hardy–Weinberg equilibrium using χ^2 tests for each locus (except *LDH-A1** and *PGM-1**). Allelic frequencies at each locus were then compared between years for each population using χ^2 tests. Differences between years were rare (see Results) so allelic counts for the 2 years were pooled and compared among populations using contingency χ^2 analysis for each locus and for all loci combined. These among-population comparisons excluded the kokanee and were performed twice, once including the collections from Baker and Cultus lakes and a second time with only the Lake Washington populations. Log-likelihood G tests (Sokal and Rohlf 1981) for all loci combined were then used to test for differences in all pairwise comparisons between populations (years pooled). The α level was set at 0.05 for statistical analyses and was then corrected for multiple comparisons (α/n, where n is the number of comparisons) in the tests for deviations from Hardy–Weinberg equilibrium ($n = 42$, $p < 0.001$), the tests for differences in allelic frequencies between years ($n = 22$, $p < 0.002$), and the pairwise G tests ($n = 21$, $p < 0.002$).

Nei's (1978) unbiased genetic distance and Cavalli-Sforza and Edwards' (1967) chord distance were calculated among the populations using data for all loci. These genetic distance measures were then used to construct a UPGMA dendrogram (Sneath and Sokal 1973) and a neighbor-joining tree (Saitou and Nei 1978), respectively.

Table 1. Tissues and buffer systems that provided the best resolution for investigating genetic variation at allozyme loci.

Enzyme	EC No.	Locus	Tissue	Buffer
Adenosine deaminase	3.5.4.4	*ADA-1**	H	TBE
Alanine aminotransferase	2.6.1.2	*ALAT**	M	TBE
Aspartate aminotransferase	2.6.1.1	*sAAT-2**	H	ACE7
Creatine kinase	2.7.3.2	*CK-A1,2**	M	TBE
		*CK-B**	M	TBE
Glucose-6-phosphate isomerase	5.3.1.9	*GPIA**	M	TBCLE
		*GPIB1,2**	M	TBCLE
L-Iditol dehydrogenase	1.1.1.14	*IDDH-1**	L	TBCL
		*IDDH-2**	L	TBCL
Lactate dehydrogenase	1.1.1.27	*LDH-A1**	M	ACE7
		*LDH-A2**	M	ACE7
		*LDH-B1**	M	ACE7
		*LDH-B2**	L	TBCL
		*LDH-C**	E	TBE
Mannose-6-phosphate isomerase	5.3.1.8	*MPI**	H	TBE
Tripeptide aminopeptidase	3.4.*.*	*PEPB1**	M	TBE
		*PEPB2**	M	TBE
Proline dipeptidase	3.4.*.*	*PEPD1**	M	TBE
Phosphoglucomutase	5.4.2.2	*PGM-1**	M	ACE7
		*PGM-2**	M	TBCLE
Superoxide dismutase	1.15.1.1	*sSOD-1**	L	TBE
Triose-phosphate isomerase	5.3.1.1	*TPI-1,2**	H	TBE

Note: Tissues are as follows: H, heart; M, skeletal muscle; E, eye; and L, liver. Buffers were made using the recipes provided in Aebersold et al. (1987).

Both cluster analyses included the sockeye salmon populations from Lake Washington and Baker Lake (years pooled), as well as the collections of kokanee from Issaquah Creek and juvenile sockeye salmon from Cultus Lake.

Fixation indices (F statistics) can provide a useful measure of the degree of population subdivision on the basis of the correlation between random gametes drawn from a population, relative to the total for all populations (Wright 1969; Chakraborty and Leimar 1987). Within Lake Washington, Wright's (1978) nonhierarchical fixation index (F_{ST}) was calculated for each highly polymorphic locus (excluding kokanee) using the formula

$$F_{ST} = \sigma_q^2/\bar{q}(1 - \bar{q})$$

where $\bar{q}$ and σ_q^2 are the interpopulation mean and variance of allelic frequencies.

F_{ST} can be used to indirectly estimate realized gene flow among populations (Wright 1969; Slatkin and Barton 1989; Cockerman and Weir 1993). This technique has been advocated for estimating levels of migration among fish populations (Chakraborty and Leimar 1987; Ryman 1991) and has been used in some studies of salmonid population structure (e.g., Berg and Gall 1988; Tallman and Healey 1994). Within Lake Washington, the average F_{ST} value for the highly polymorphic loci was used to estimate realized gene flow ($N_e m_e$) among the sockeye salmon populations using the formula

$$F_{ST} = \frac{1}{1 + 4\,N_e\,m_e\left(\frac{n}{n-1}\right)^2}$$

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Table 2. Variant allelic frequencies and sample sizes for highly polymorphic loci ($q > 0.05$).

		*ALAT**			*PGM-1**		*PGM-2**		*LDH-A1*	
Population	Year	*N*	**91*	**95*	*N*	**NULL*	*N*	**136*	*N*	**500*
Baker Lake	1992	40	0.563	0.075	39	0.320	56	0.170	56	0.000
	1993	43	0.512	0.047	40	0.387	64	0.117	64	0.000
	Total	83	0.536	0.060	79	0.356	120	0.142	120	0.000
Cultus Lake	1992	40	0.050	0.000	40	0.962	40	0.175	64	0.000
Lake Wash. Beach	1992	40	0.400	0.075	40	0.387	41	0.037	41	0.156
	1993	40	0.275	0.050	39	0.599	39	0.051	40	0.000
	Total	80	0.338	0.063	79	0.503	80	0.044	81[b]	0.111
Bear Creek	1992	40	0.225	0.150	40	0.671	63	0.127	40	0.224
	1993	43	0.279	0.163	40	0.592	52	0.154	12	0.408
	Total	83	0.253	0.157	80	0.632	115	0.139	52	0.277
Cedar River	1992	76	0.382	0.072	40	0.447	134	0.086	135	0.086
	1993	115	0.361	0.074	40	0.354	115[a]	0.096	117	0.000
	Total	191	0.369	0.073	80	0.403	249	0.090	252[b]	0.063
Cottage Creek	1992	40[a]	0.375	0.113	40	0.632	52	0.144	40	0.158
	1993	40	0.213	0.088	40	0.689	40	0.138	38	0.281
	Total	80	0.294	0.100	80	0.661	92	0.141	78	0.226
Issaquah Creek	1992	40	0.313	0.063	40	0.224	83	0.060	40	0.000
	1993	60	0.317	0.067	40	0.316	64	0.047	83	0.000
	Total	100	0.315	0.065	80	0.274	147	0.054	123	0.000
kokanee	1993	16	0.357	0.071	16	0.433	16[a]	0.250	16	0.559

Note: The frequency of the **100* allele is not shown but is equal to 1 minus the frequency of the other allele(s). Allelic frequencies for *PGM-1** and *LDH-A1** were determined by the square root of the frequency of null homozygotes (assuming Hardy–Weinberg equilibrium).

[a]Collections for which allelic frequencies, at a given locus, deviated from Hardy–Weinberg equilibrium ($p < 0.05$).

[b]Populations for which allelic frequencies, at a given locus, differed between years ($p < 0.05$).

where N_e is the effective population size, m_e is the effective rate of migration, and n is the number of populations (Chakraborty and Leimar 1987).

All statistical analyses were performed using three computer programs. BIOSYS-1 (release 1.7, Swofford and Selander 1981) was used to test for deviations from Hardy–Weinberg equilibrium; to calculate F_{ST} values, Nei's (1978) unbiased genetic distances, and Cavalli-Sforza and Edwards' (1967) chord distances; and for the construction of the UPGMA dendrogram. PHYLIP (version 3.57c; J. Felsenstein, University of Washington, Department of Genetics, Box 357360, Seattle, WA 98195-7360, U.S.A.) was used to construct the neighbor-joining tree. A *G* test program (David Teel, National Marine Fisheries Service, Coastal Zone and Estuarine Studies Division, Northwest Fisheries Science Center, NOAA, 2725 Montlake Boulevard East, Seattle, WA 98112, U.S.A.) was used for the pairwise comparisons between populations.

Results

Variation within populations

Of the 22 loci, four (*ALAT**, *PGM-1**, *PGM-2**, and *LDH-A1**) were highly polymorphic (Table 2), three (*LDH-B2**, *GPI-B1,2**, and *TPI-1.2**) exhibited low polymorphism, and the remainder were monomorphic. Variant alleles for the loci at which polymorphism was low included *LDH-B2*110* (found at a frequency of 0.013 in each of the Cedar River, Cottage Creek, Cultus Lake, and beach populations), *LDH-B2*85* (found in the beach (0.038), Cedar River (0.050), Cottage Creek (0.025), and Issaquah Creek (0.025) populations), *GPI-B1,2*13* (found in the Bear Creek (0.005), Cedar River (0.003), and Cottage Creek (0.003) populations), and *TPI-1.2*-54* (found at a frequency of 0.006 in the Cottage Creek and Issaquah Creek populations).

Only one of the allelic variants detected in this study had not been described previously in the literature. This *LDH-A1** variant was a phenotypic analog to a similar polymorphism described in brown trout, *Salmo trutta* (Allendorf et al. 1984), where the variant allele either synthesizes a homotetrameric band that comigrates with the homotetramer for the common allele of *LDH-A2** or is not synthesized (i.e., a null allele). For this polymorphism, the four-banded phenotype of the heterozygote cannot be consistently distinguished from the five-banded phenotype of the common homozygote. However, the frequency of the allele can be estimated from the frequency of the homozygous variant phenotype (assuming Hardy–Weinberg proportions). We interpreted the variant as an active allele rather than a null on the basis of variation in band intensity for each phenotype (P. Aebersold and G. Winans, National Marine Fisheries Service, Coastal Zone and Estuarine Studies Division, Northwest Fisheries Science Center, NOAA, 2725 Montlake Boulevard East, Seattle, WA 98112, U.S.A., unpublished data). The allele was designated *LDH-A1*500* on the basis of its mobility in the buffer system that we used. In a TBCLE type buffer system, it had a mobility of **86*.

Tests for genotypic variation within collections and for year-class variation within each population generally conformed to values expected from random breeding populations.

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Table 3. Pairwise comparisons of genetic relationships among sockeye salmon populations from Lake Washington, Baker Lake, and Cultus Lake.

	Population						
Site	Cedar	Issaquah	Bear	Cottage	Beach	Baker	Cultus
Cedar		0.001	0.005	0.005	0.000	0.002	0.023
Issaquah	39.4[a] (8)		0.011	0.010	0.003	0.003	0.029
Bear	68.4[a] (8)	137.1[a] (7)		0.000	0.003	0.011	0.014
Cottage	59.3[a] (8)	133.5[a] (7)	5.7 (8)		0.002	0.010	0.011
Beach	14.0 (8)	52.3[a] (6)	37.2[a] (7)	29.6[a] (7)		0.004	0.017
Baker	51.9[a] (8)	35.1[a] (5)	134.4[a] (7)	127.4[a] (7)	70.4[a] (6)		0.032
Cultus	162.8[a] (8)	174.6[a] (6)	127.2[a] (8)	109.3[a] (7)	135.4[a] (7)	181.7[a] (5)	

Note: Nei's (1978) unbiased genetic distance is above the diagonal and *G* test values from pairwise comparisons are below the diagonal. The degrees of freedom for the *G* tests are given in parentheses. Corrections for multiple comparisons (α/n, where $\alpha = 0.05$ and $n = 21$) yield the final significance level ($p < 0.002$).

[a] $p < 0.002$.

Of the 42 tests for Hardy–Weinberg proportions, three deviated from expectations at $\alpha = 0.05$ (Table 2). After correction for multiple comparisons ($p < 0.001$), only two (*PGM-2** at the Cedar River in 1993 and *ALAT** at Cottage Creek in 1992) were significant. In both cases, the deviation was due to a deficiency of heterozygotes.

Only 2 of the 24 between-year comparisons (six populations at each of the four highly polymorphic loci) showed a significant difference in allelic frequencies. After correction for multiple comparisons ($p < 0.002$), both of these (*LDH-A1** at the Cedar River and the beach) remained significant. Presumably, this year-class variation reflected the relative insensitivity of estimating allelic frequencies from the square root of the frequency of rare homozygotes (both populations had one fish that was homozygous for the **500* allele in 1 year and no such fish in the other year). Owing to the general consistency in allelic frequencies between years, data for the 2 years at each location were pooled for each locus to facilitate comparisons among the populations.

Variation among populations

Analysis of allelic variation among the populations was based primarily on the four highly polymorphic loci because allelic variants (i.e., other than the **100*) at the less polymorphic loci were only represented by a single heterozygous individual in a few populations. For the first set of interpopulation comparisons (Baker Lake, Cultus Lake, and the five Lake Washington sockeye salmon populations), significant genetic differences were present at each locus (*ALAT**, $\chi^2 = 95.79$, df = 12, $p < 0.001$; *PGM-1**, $\chi^2 = 148.91$, df = 6, $p < 0.001$; *PGM-2**, $\chi^2 = 28.62$, df = 6, $p < 0.001$; *LDH-A1**, $\chi^2 = 168.24$, df = 6, $p < 0.001$) and when data for all loci were combined ($\chi^2 = 457.37$, df = 48, $p < 0.001$). For the second set of interpopulation comparisons (just the five Lake Washington sockeye salmon populations), significant genetic differences remained at each locus (*ALAT**, $\chi^2 = 19.23$, df = 7, $p = 0.014$; *PGM-1**, $\chi^2 = 66.61$, df = 4, $p < 0.001$; *PGM-2**, $\chi^2 = 20.87$, df = 4, $p < 0.001$; *LDH-A1**, $\chi^2 = 110.00$, df = 4, $p < 0.001$) and when data for all loci were combined ($\chi^2 = 226.58$, df = 36, $p < 0.001$).

Pairwise *G* tests (using all loci) revealed significant differences in allelic frequencies between all possible population pairs except for Bear Creek versus Cottage Creek and Cedar River versus the beach (Table 3). The highest levels of significance

Fig. 2. UPGMA dendrogram for all populations based on pairwise comparisons of Nei's (1978) unbiased genetic distance averaged over 6 polymorphic loci and 16 monomorphic loci.

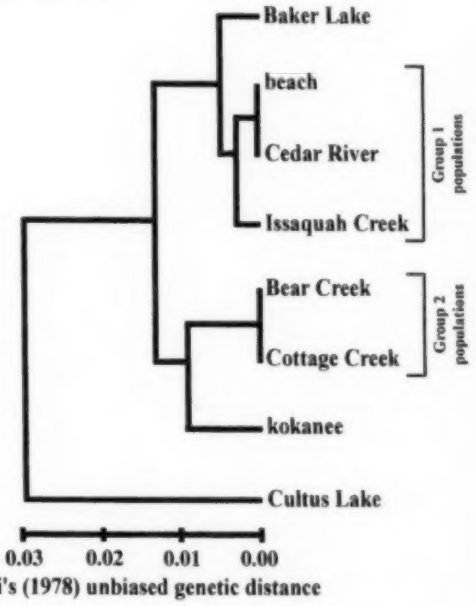

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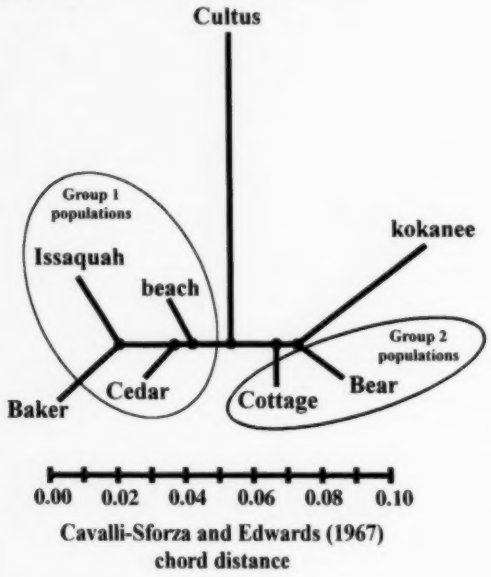
Fig. 3. Neighbor-joining tree (Saitou and Nei 1987) for all populations based on pairwise comparisons of Cavalli-Sforza and Edwards' (1967) chord distance averaged over 6 polymorphic loci and 16 monomorphic loci.



were for the comparisons that involved the Cultus Lake fish. The patterns revealed by pairwise *G* tests matched those revealed by genetic distance measures (Table 3).

The UPGMA projection of Nei's (1978) unbiased genetic distance revealed several major distinctions among the populations (Fig. 2). The most divergent population, sockeye salmon from Cultus Lake, differed from populations at all of the other locations at a mean relative genetic distance of about 0.025. The other populations grouped into two distinct clusters, separated by a genetic distance of about 0.014. One cluster contained collections of sockeye salmon from Baker Lake and three of the Lake Washington populations (group 1 populations: Cedar River, Issaquah Creek, and the beach). The other cluster was comprised of sockeye salmon from Bear Creek and Cottage Creek (group 2 populations) and of kokanee from Issaquah Creek. The general pattern revealed by the UPGMA dendrogram was similar to that obtained using a neighbor-joining tree with Cavalli-Sforza and Edwards' (1967) chord distances (Fig. 3).

Different loci were involved in distinguishing among the various populations. Cultus Lake fish stood apart with near fixation of the *ALAT*100* and *PGM-1*NULL* alleles (see also Wood et al. 1994). The two major clusters that contained Lake Washington populations were best distinguished from each other by allelic frequency variation at *LDH-A1* and, to a lesser degree, at *PGM-1**. Within the first population cluster (Baker Lake, Cedar River, Issaquah Creek, and the beach), frequencies of the *ALAT*100* and the *PGM-1*NULL* alleles distinguished between the Issaquah Creek fish and those from Cedar River and the beach (Table 2). Within the other population cluster (Bear Creek, Cottage Creek, and kokanee), kokanee differed markedly from the two sockeye salmon populations

Table 4. Documented levels of genetic differentiation within sockeye salmon lake systems.

		F_{ST}			
Lake	Location	*PGM-1**	*PGM-2**	*ALAT**	*LDH-A1**
Washington	Washington	0.089	0.012	0.006	0.144
Nachiki	Russia	—	0.007	—	—
Kuril	Russia	0.029	0.012	0.006	—
Dvu-Yurta	Russia	—	0.006	—	—
Clark	Alaska	0.008	0.010	0.025	—
Iliamna	Alaska	0.005	0.008	0.017	—
Karluk	Alaska	—	0.003	—	—
Meziadin	B.C.	0.010	0.006	0.004	—
Babine	B.C.	0.006	0.003	0.010	—
Shuswap	B.C.	0.054	0.009	—	—

Note: For Lake Washington, Wright's (1978) nonhierarchical fixation index (F_{ST}) was calculated for each highly polymorphic locus using BIOSYS-1 (Swofford and Selander 1981). Values for the other lake systems are from Varnavskaya et al. (1994*b*).

at *LDH-A1** and to a lesser degree at *PGM*1* and *PGM*2* (Table 2).

Fixation indices, calculated for sockeye salmon within the Lake Washington drainage, identified varying degrees of differentiation indicated by allelic variation at the different loci (Table 4). The higher values for *LDH-A1** and *PGM-1** reflected their broader range of allelic frequencies within the drainage and contrasted with the lower F_{ST} values and more limited allelic frequency ranges of *ALAT** and *PGM-2**. The average F_{ST} value for the highly polymorphic loci was 0.057, equivalent to an estimate of 2.64 effective migrants per generation (i.e., realized gene flow among the populations).

Discussion

The use of aliozymes to infer ancestral origins and reproductive isolation assumes that electrophoretically detectable genetic variation is predominantly neutral with respect to selection (Allendorf and Phelps 1981; Utter 1991). The applicability of this assumption to the present study deserves consideration. For selection to override the effects of genetic drift, the coefficient of selection (s) would need to be considerably greater than the reciprocal of the effective population size, N_e (Chakraborty and Leimar 1987, p. 118). N_e was not estimated for the Lake Washington populations but the absolute number of spawners (N) has been at least as low as 100 for the beach (several years), 707 for Issaquah Creek (1990), 1795 for the Bear–Cottage system (1989), and 76 000 for the Cedar River (1993; Ron Egan, unpublished data). In each case, N_e would likely be much smaller than N owing to fluctuations in population size, unequal sex ratios, overlapping generations, and nonrandom mating (Hartl and Clark 1989), all of which are common in salmon populations. Some studies have reported a small apparent fitness advantage for individuals that were heterozygous at certain loci (e.g., Altukhov and Salmenkova 1991). However, genotype frequencies for the Lake Washington populations rarely deviated from Hardy–Weinberg expectations and the few that did were characterized by a heterozygote deficit. Hence, it is unlikely that $s \gg 1/N_e$ and effective neutrality was probably a valid assumption in the present study.

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Ancestral origins

Two genetically divergent population groups were identified within Lake Washington (Figs. 2 and 3). One of these groups was comprised of sockeye salmon from the Cedar River, Issaquah Creek, and the beach (group 1), and the other was comprised of sockeye salmon from Bear Creek and Cottage Creek (group 2). This general pattern has been corroborated by independent processing, scoring, and statistical analysis using the fish collected in this study and additional fish collected from Lake Washington in 1994 (G. Winans and P. Aebersold, unpublished data). In the present study, the group 1 and group 2 populations were distinguishable from each other at several loci (Table 2), but most conspicuously at *LDH-A1**. In the independent analysis mentioned above, a larger suite of allozyme loci were used, revealing that the distinction between group 1 and group 2 populations extended to a number of other polymorphic loci not reported here (G. Winans and P. Aebersold, unpublished data).

The populations in group 1 showed a genetic affinity to fish collected from Baker Lake (Figs. 2 and 3). Thus, in conjunction with information on hatchery supplementation and the distribution and intensity of stocking efforts (see Introduction), the genetic data supported the inference that transplants from Baker Lake made a strong contribution to the Cedar River, Issaquah Creek, and beach populations. In contrast, the populations in group 2 were not closely related to either of the major donor populations (Baker Lake or Cultus Lake). In fact, they were more similar to Issaquah Creek kokanee than they were to the other Lake Washington sockeye salmon populations (Figs. 2 and 3). Hence, the Bear Creek and Cottage Creek populations do not appear to have been derived from any of the known introductions into the Lake Washington drainage. We infer that these fish represent native stocks that somehow persisted, albeit at low levels, in the Sammamish River tributaries despite considerable anthropogenic disturbances elsewhere in the watershed.

Other than Baker Lake, two non-native sources could conceivably have contributed to the Lake Washington gene pool. The contribution of the first source (in 1917) could not be evaluated using genetic techniques because its origin was unknown. However, success for this early transplant was unlikely because (*i*) it coincided with the diversion of Cedar River and the construction of the Lake Washington ship canal, (*ii*) only one introduction was made, and (*iii*) it was directly into the lake rather than a spawning stream (Ajwani 1956; Woodey 1966; Hendry 1995). Furthermore, introgression between native fish and returns from the transplant was unlikely because populations of anadromous sockeye salmon within the watershed were very small. Another possible source of non-native fish in the drainage was Cultus Lake (Woodey 1966; Seeb and Wishard 1977). In the present study, Cultus Lake fish were genetically distinct from all of the Lake Washington populations (Figs. 2 and 3), indicating that transplants from this location probably made little contribution to the existing Lake Washington gene pool. Nevertheless, frequencies for some alleles in the Lake Washington populations (particularly *ALAT*91* and *PGM-1*NULL*) were intermediate to those found at Baker Lake and Cultus Lake (Table 2). Therefore, we do not completely discount the possibility that Cultus Lake fish made some contribution to the Lake Washington sockeye salmon populations.

Within group 1, the three Lake Washington populations were more similar to each other than they were to the Baker Lake populations (Fig. 2). This pattern could have arisen for several reasons. First, the fish introduced to Lake Washington may not have been representative of the Baker Lake population at that time. This was unlikely because more than 3 million fry and fingerlings were introduced in a total of nine transplants over 5 years (Hendry 1995). Second, the Baker Lake fish, and (or) those transplanted to Lake Washington, could have introgressed with fish of another origin (see above). Introgression probably contributed to the divergence of the Lake Washington populations because both native and introduced fish persisted within the watershed. Third, genetic drift during the 47 years of isolation (1945–1992) could have resulted in the divergence of the ancestral and derived populations. Theoretically, this length of time (12 generations) would be sufficient for the observed level of divergence in the absence of selection or migration (Allendorf and Phelps 1981). Moreover, these populations have undergone several periods of low abundance (recently for Baker Lake and during the 1940s and 1950s for Lake Washington), which would have increased genetic drift and hence accelerated population divergence in a situation analogous to that postulated for pink salmon (*Oncorhynchus gorbuscha*) in the Great Lakes (Gharrett and Thomason 1987).

Population differentiation

The level of population differentiation, as measured by F_{ST}, was larger within Lake Washington than that documented within a number of other sockeye salmon lake systems (Table 4). This differentiation was reflected in significant genetic differences among many of the populations (Table 3). Some of this interpopulation genetic diversity was likely initiated by their multiple origins (i.e., Baker Lake or native) but subsequent gametic exchange among the populations has been insufficient to homogenize allelic frequencies. Therefore, natal homing has probably contributed to the differentiation of the various Lake Washington populations. However, significant genetic differences should not necessarily be interpreted as a complete lack of migration (Allendorf and Phelps 1981; e.g., Berg and Gall 1988). Accordingly, realized gene flow among the Lake Washington populations was estimated to be about 2.64 migrants per generation.

Estimates of gene flow from F_{ST} are subject to several assumptions that can limit their applicability in certain systems (Chakraborty and Leimar 1987; Ryman 1991). For instance, the calculation of $N_e m_e$ from F_{ST} assumes genetic and demographic equilibrium (Wright 1969; Slatkin 1994). For Lake Washington, the introductions of Baker Lake fish occurred less than 15 generations ago, a time frame where an equilibrium may not yet have been reached (Takahata 1983; Slatkin 1993). The possible violation of the equilibrium assumption dictates that 2.64 migrants per generation should be considered approximate. Nevertheless, the large genetic difference among several populations, the relatively high F_{ST}, and the indication that migration was restricted all attest to substantial reproductive isolation for several Lake Washington populations.

The greatest variation in allelic frequencies within the watershed was evident between the group 1 and group 2 populations. Within group 1, sockeye salmon from Issaquah Creek

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were also distinguishable from those in Cedar River and from the beach (Table 3). This finding accords with that of Seeb and Wishard (1977), who reported genetic differences between sockeye salmon collected in Lake Sammamish and those collected in the Cedar River. Thus, there is evidence for at least three genetically distinct populations within Lake Washington: (*i*) Cedar River and the beach, (*ii*) Issaquah Creek, and (*iii*) Bear and Cottage creeks.

Allelic variation was not sufficient to distinguish between sockeye salmon from Bear and Cottage creeks or between the Cedar River and beach populations. The lack of a detectable difference in the former comparison was not unexpected because of the geographic proximity of the two creeks (<1 km) and the similar life histories of their sockeye salmon populations (Hendry and Quinn 1996). Conversely, the similarity of the Cedar River and the beach collections was surprising because other studies using allozymes (Seeb and Wishard 1977) and life history traits (Woodey 1966; Hendry and Quinn 1996) provided evidence that these populations were distinct. For example, Seeb and Wishard (1977) reported that the frequency for the *PGM-2*136* allele was 0.158 for Cedar River sockeye salmon but only 0.015 in beach fish. In the present study, this allele also occurred more frequently in the Cedar River population but the difference was smaller (0.090 vs. 0.044).

Allelic frequencies were stable in the Cedar River population but varied between years at the beach for two of the highly polymorphic loci (*ALAT** and *PGM-1**; Table 2). Temporal variation at the beach probably resulted from two factors. First, small population sizes (Woodey 1966; Ron Egan, unpublished data) would magnify the effects of genetic drift, and second, the small sample sizes would reduce the precision of allelic frequency estimates and the power of statistical tests (Utter et al. 1987). Thus, two competing hypotheses exist for the apparent similarity of the Cedar River and the beach populations. One is that straying from Cedar River, which usually has a population more than three orders of magnitude larger than the beach, may have had a homogenizing influence. Alternatively, the small N_e and small sample sizes at the beach may have limited the ability to detect differences.

Conclusions

Both native and introduced sockeye salmon contributed to the existing Lake Washington population mixture. The contribution of each lineage varied among the different spawning sites in proportion to the location and intensity of stocking. For instance, numerous transplants were made into Cedar River and into Issaquah Creek, populations that genetic analysis revealed to be primarily of non-native origin. In contrast, few transplants occurred into Bear and Cottage creeks, which appeared to have fish primarily of native origin. The intensity of stocking may also have contributed to the success of the Baker Lake transplants, relative to those from Cultus Lake, because many more fish were introduced from the former location. Nevertheless, the introductions from Cultus Lake were probably sufficient to produce some immediate returns to the watershed, thereby providing the potential for introgression with populations of native or Baker Lake origin. Consistent with this possibility, allelic frequencies in some Lake Washington populations were intermediate to those found in the Baker Lake and Cultus Lake populations.

Variation in life history, morphology, and behavior may also have contributed to the differential success of the two major donor groups. The sockeye salmon in Cultus Lake spawned primarily on lake beaches while those from Baker Lake spawned predominantly in tributary streams (Hendry and Quinn 1996). Presumably, the Baker Lake fish were adapted for reproduction in stream environments whereas the Cultus Lake fish were not. If this were true, the fitness of salmon transplanted from Baker Lake might have been higher than that of salmon transplanted from Cultus Lake because all of the introductions occurred into stream environments (primarily Cedar River and Issaquah Creek).

Among the populations derived primarily from Baker Lake, significant genetic differences were present. This result may reflect population divergence after the introduced populations became reproductively isolated from each other. The three derived populations also differed from the ancestral group. This difference between derived and ancestral populations could have resulted from introgression with native fish or with the fish introduced from Cultus Lake. Alternatively, it could indicate genetic divergence after the cessation of gene flow from Baker Lake into the Lake Washington drainage. Therefore, some evidence indicated two patterns of possible divergence: (*i*) between ancestral and derived groups, and (*ii*) among derived populations of the same lineage. The Lake Washington populations have only been completely isolated from the Baker Lake population since 1945 (12 generations). If the differences that arose during this time can be attributed to genetic drift, this study provided initial documentation of the rapidity with which neutral evolution can occur in Pacific salmon.

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Isolation and characterization of novel salmon microsatellite loci: cross-species amplification and population genetic applications

Kim T. Scribner, Judy R. Gust, and Raymond L. Fields

Abstract: Twenty-two variable number of tandem repeat microsatellite dinucleotide repeat ($[GA]_n$ and $[CA]_n$) loci were cloned from sockeye salmon (*Oncorhynchus nerka*) partial genomic libraries. Characteristics and optimal polymerase chain reaction (PCR) conditions were defined for each locus. The degree of conservation of sequences flanking microsatellite repeat motifs and the utility of heterologous PCR primers for analyses in closely related taxa was tested using 10 salmonid species from four genera. Nearly all microsatellite primers produce amplification products in multiple species, suggesting broad application in salmonid research. The utility of these loci for population genetic studies was tested using individuals ($N = 83$) from three spawning populations of chinook salmon (*Oncorhynchus tshawytscha*) from the Yukon River, Yukon Territories. Twelve of 16 loci screened were polymorphic (mean heterozygosity = 0.254). Genetic distance estimates between populations were concordant with results from a previous allozyme survey of these same populations. Discussions of the utility of microsatellite markers in salmonid population genetic research are presented in light of recently described statistical methodologies based on mutational properties and interallelic differences in repeat score.

Résumé : À partir de banques génomiques partielles de saumons sockeye (*Oncorhychus nerka*), on a cloné 22 loci de microsatellite séquences répétées en tandem dinucléotidiques ($[GA]_n$ et $[CA]_n$). Les caractéristiques et les conditions optimales pour la PCR ont été définies pour chaque locus. Le degré de conversion des séquences qui flanquent les motifs répétés des microsatellites et l'utilité des amorces hétérologues de la PCR pour des analyses dans des taxons étroitement apparentés ont été évalués avec 10 espèces de salmonidés appartenant à quatre genres. Presque toutes les amorces de microsatellites ont donné des produits d'amplification chez plusieurs espèces, ce qui laisse supposer qu'elles sont applicables à grande échelle dans les recherches sur les salmonidés. On a évalué l'utilité de ces loci dans les études génétiques des populations, en utilisant des individus ($N = 83$) provenant de trois populations de saumons quinnat (*Oncorhychus tshawytscha*) en cours de reproduction, vivant dans le fleuve Yukon (Territoires du Nord-ouest). Douze des 16 loci examinés étaient polymorphes (hétérozygotie moyenne = 0,254). Les estimations de distance génétique entre les populations concordaient avec les résultats obtenus au cours d'une étude allozymatique précédente des mêmes populations. On examine l'utilité de marqueurs à microsatellites dans les recherches génétiques de populations de salmonidés, à la lumière de méthodes statistiques récemment décrites, basées sur les propriétés mutationnelles et les différences inter-alléliques des résultats obtenus avec les séquences répétées.
[Traduit par la Rédaction]

Introduction

Characterization and quantification of genetic diversity has long been a major goal in both basic and applied fisheries research. For many years, protein allozyme electrophoresis has provided much of the empirical population-level data (Allendorf et al. 1987; Gharrett and Smoker 1994). However, recent advances in molecular technology have dramatically increased the number of genetic markers capable of resolving variation at the DNA level (see review by Park and Moran 1994). Many studies have utilized maternally inherited mitochondrial DNA (e.g., surveys summarized in Avise 1992), and an increasing number of nuclear genetic markers have been developed, including restriction fragment length polymorphism (RFLP) analysis of single-copy nuclear DNA (Wirgin and Maceda 1991), coding genes such as lactate dehydrogenase-B (Crawford et al. 1989), single locus minisatellites (Taggart and Ferguson 1990; Bentzen et al. 1991; Prodöhl et al. 1994), multi-locus DNA minisatellites (Prodöhl et al. 1992), and microsatellites (Estoup et al. 1993).

Despite the rapid development and growing interest in the use of molecular genetic markers for population-level analyses, few studies have addressed the utility of a single class of nuclear markers across a range of species, nor have results of molecular markers been compared with results using more widely employed techniques (i.e., allozymes). In addition, little is known of levels of genetic variability within and among populations at the DNA level.

One class of markers that is receiving increasing attention is that of variable number of tandem repeat (VNTR) microsatellite loci (Tautz 1989; Bruford and Wayne 1993).

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K.T. Scribner,[1] J.R. Gust,[2] and R.L. Fields. Alaska Science Center, National Biological Service, 1011 E Tudor Road, Anchorage, AK 99503, U.S.A.

[1] Author to whom correspondence should be addressed. e-mail: Kim_Scribner@NBS.Gov
[2] Present address: Department of Biological Sciences, University of Alaska–Anchorage, Anchorage, AK 99508, U.S.A.

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Microsatellites consist of tandem arrays of short (generally ≤5 bp) repeat motifs such as $[CA]_n$ or $[AAT]_n$. Many simple-sequence motifs occur in extremely high frequency in eukaryotic genomes (e.g., every 30 kb for $[GT]_n$ repeats in mammals; Stallings et al. 1991) relative to random motifs of equivalent length. High frequency of occurrence and uniformity of distribution within most eukaryotic genomes (Luty et al. 1990), and high levels of variation (e.g., Amos et al. 1993), have fostered a growing appreciation of their use in genome mapping (Dietrich et al. 1992), paternity (Morin et al. 1994), and forensics (Jeffreys et al. 1992). These markers have increasingly been used in population studies of both contemporary (e.g., Scribner et al. 1994; Taylor et al. 1994; Slatkin 1995) and historical (Ellegren 1991) samples. Microsatellites are particularly amenable for population-level analyses that necessitate the characterization of many individuals. Loci are assayed using polymerase chain reaction (PCR)-based techniques, in which PCR primers are designed from sequences flanking the repeat motif, and amplification products are run on denaturing polyacrylamide gels. Further, microsatellites that have been characterized in specific taxa have proved to be homologous and polymorphic in a diverse number of species of varying degrees of relatedness (Moore et al. 1991; Schlotterer et al. 1991).

In this study we present data from a series of novel dinucleotide ($[GA]_n$ and $[CA]_n$) microsatellite loci that were developed from sockeye salmon (*Oncorhynchus nerka*). Optimal PCR conditions are described and results of cross-species amplifications are presented in the hope of stimulating interest in the use of these markers for future salmonid research. The utility of these markers for population genetic studies was tested using a *Oncorhynchus tshawytscha* data set from tributaries of the Yukon River. Levels of interpopulation heterogeneity in allele frequency are compared with a previous allozyme study of these same populations.

Methods and materials

Sampling locations

Samples of adult chinook salmon were collected from specific spawning grounds on the North Fork of the Klondike River ($N = 29$), McQuesten River ($N = 27$), and Stoney Creek of the Takhini River ($N = 27$) during August 23–30, 1990. Tissues were taken in the field and placed immediately into liquid N_2. Samples were subsequently stored at –70°C in the laboratory until DNAs were extracted. Samples of the remaining nine species used in cross-species amplifications were as follows: *Oncorhynchus kisutch*, Kenai River, Alaska; *Oncorhynchus gorbuscha*, Kenai River, Alaska; *Oncorhynchus keta*, Fishing Branch of the Porcupine River, Yukon Territory; *O. nerka*, Kenai River, Alaska; *Oncorhynchus mykiss*, Kenai River, Alaska; *Oncorhynchus clarki*, brood stock from Montana State University; *Thymallus arcticus*, Coleville River, Alaska; *Salvelinus malma*, Kenai River, Alaska; and *Salmo salar*, Dee River, Scotland, U.K.

DNA extractions

DNA was extracted using standard proteinase K, phenol–chloroform techniques (Sambrook et al. 1989; Bruford et al. 1992) and resuspended in TE (10 mM Tris–HCl, pH 8.0, 1 mM EDTA). DNA concentrations were determined using fluorometry, and working stocks of 50 ng/mL were made for each sample. One hundred nanograms of DNA were used for each PCR reaction (see below).

Cloning and characterization of microsatellite loci

Two procedures were used for library construction and screening of microsatellites. Initial libraries were made using a modification of procedures described in Rassmann et al. (1991). In brief, *O. nerka* genomic DNA was isolated as above and was completely digested using three restriction enzymes (*Alu*I, *Rsa*I, and *Hae*III). The 300- to 700-bp size fraction was isolated using an electroelution procedure (Bruford et al. 1992), and was ligated into a *Sma*I digested, dephosphorylated pUC18 (Stratogene) plasmid (50 ng genomic DNA to 200 ng vector). The ligation reaction was transformed using electroporation into *E. coli* JS5 (BioRad, Richmond, Calif.), which were plated at low density on agar containing IPTG/X-gal and ampicillin (100 mg/mL). Filter replicates of each plate were made using Hybond-Nfp (Amersham Corp., Arlington Heights, Ill.) nylon membranes. Membranes were probed using [γ-^{32}P]ATP end-labeled simple-sequence polymers ($[GA]_{20}$ and $[CA]_{20}$). Positive clones were identified by autoradiography. Putative positive recombinant plasmid DNAs were purified using miniprep columns (Promega, Madison, Wis.) and transferred to Nytran (Schleicher and Schuell, Keene, N.H.) membranes using a 96-well dot-blot apparatus (BioRad). A second screening was performed that included oligo $[GA/CT]_n$ and $[CA/GT]_n$ standards (Pharmacia, Alameda, Calif.) as positive controls. Positives from the second screening were sequenced using modifications of standard di-deoxy double-stranded Sequenase (USB) protocols, including DMSO (Winship 1989).

Greater efficiency, in terms of the number of positives identified per unit effort, was obtained using a modification of library enrichment procedures described in Ostrander et al. (1992). Genomic DNA was completely digested using the restriction enzyme *Sau*3AI, size-selected as described above, and ligated into a dephosphorylated pBluescript KS+ (Strategene) phagemid vector. Portions of the ligation mixture were transformed using electroporation into *E. coli* CJ236 (BioRad) deficient in dUTPase (*dut* gene product) and uracil-*N*-glycosylase (*ung* gene product). Colonies were plated at high density on large (150 × 15 mm) LB plates containing ampicillin (100 mg/mL). After overnight incubation, colonies were washed with 10 mL of 2× YT media (Sambrook et al. 1989), after which cell suspensions were collected. One hundred millilitres of washed cells were used to inoculate an overnight culture, which was grown to saturation. Each of 24 tubes (2 mL of 2× YT media) was inoculated with 100 µL of overnight culture and infected with M13KO7 helper phage. Single-stranded DNA was prepared using PEG precipitation (Sambrook et al. 1989), and primer extension reactions were carried out by combining 3 µg of single-stranded DNA, 20 pmol of $[CA]_{20}$ or $[GA]_{20}$ primer, 10 µL of 10× PCR buffer (Perkin Elmer, Branchburg, N.J.), 200 µM each dNTP in a final volume of 100 µL using conditions specified in Ostrander et al. (1992). Primer extension reactions were terminated by extracting the mixture using one volume of phenol – chloroform – isoamyl alcohol (25:24:1) followed by extraction using chloroform –isoamyl alcohol (24:1). DNA was precipitated in the presence of salt (0.1 volume 3 M sodium acetate, pH 5.5) and glycogen (2 µL of 1 mg/mL) using 100% ethanol, followed by washing in 70% ethanol. Precipitates were resuspended in 1× TE and incubated overnight at 4°C with T4 ligase under conditions specified by the manufacturer (Pharmacia). The ligation mix was heat-inactivated for 10 min at 65°C, cleaned using phenol–chloroform, precipitated as above, and resuspended in 15 µL H_2O. One to three microlitres of the resuspended double-stranded DNAs were transformed using electroporation into *E. coli* MV1109 (BioRad) and plated at low density. Colony screening was preformed as described in Ostrander et al. (1992), and a second screening was conducted as above. Positive colonies were isolated and DNA was prepared as above. Double-stranded sequencing was performed for all positive colonies using standard Sequenase (USB) procedures, including DMSO as above.

Development of PCR primers and primer optimization

Primer sequences within flanking regions adjacent to microsatellite

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loci were developed using the software OLIGO (Rychlik and Rhoads 1989). PCR conditions for each primer pair were optimized using sockeye and chinook genomic DNAs using a 30-μL reaction mix consisting of 10 pmol of each primer, dNTPs at 200 μmol each, 0.25 units *Taq* DNA polymerase (Cetus), and PCR buffer (10 mM Tris–HCl, pH 8.3, 1.5 mM $MgCl_2$, 50 mM KCl, 0.01% gelatin, 0.01% NP–40, 0.01% Triton X–100) for 30–35 cycles. Products were visualized on 1.5% agarose gels using ethidium bromide staining. Primer sequences and annealing temperatures are provided in Table 1.

Chinook population samples were screened for variation using [γ-^{32}P]ATP end-labeled primers. One primer from each pair was end-labeled using T4 polynucleotide kinase according to manufacturer's specifications (Pharmacia). PCR reactions were as described above (using locus-specific annealing temperatures; Table 1) except that concentrations of both primers and dNTPs were reduced to one-tenth. The entire PCR reaction was mixed with 12 μL of formamide loading dye (95% formamide, 20 mM EDTA, 0.05% bromphenol blue, 0.05% xylene cyanol) and heated for 5 min at 95°C before loading onto a 6% denaturing sequencing gel. An M13 control sequencing reaction (USB sequence kit) was run adjacent to the samples to provide an unambiguous size marker for the microsatellite alleles. Gels were dried and autoradiographed overnight at –70°C using intensifying screens.

Analysis of microsatellite polymorphisms

Levels of genetic variation for each of three chinook salmon populations was assessed as the mean number of alleles per locus (A), observed heterozygosity (H_{DC}), and expected (under Hardy–Weinberg) heterozygosity (H_E). Deviations from Hardy–Weinberg equilibrium were tested for each variable locus using χ^2 tests with pooling (Hartl and Clarke 1988) to account for the presence of many rare alleles. The variance in allele frequency among populations was tested using θ as described by Weir and Cockerham (1984), and significance of θ was tested by jackknifing across samples (Weir 1990). Nei's genetic distance (Nei 1972) and recently defined distance measures (Δ, Goldstein et al. 1995; D_{AS}, Bowcock et al. 1994) based on population allele frequencies and allele repeat scores were calculated for all pairwise population comparisons. Measures of interpopulation genetic distance were compared with estimates for these sample populations based on an allozyme survey (22 loci) conducted previously (Wilmot et al. 1992). Interpopulation distances were further summarized in the form of UPGMA phenograms. Estimates of allele frequency, Nei's genetic distance, and heterozygosity were calculated using the BIOSYS–1 program (Swofford and Selander 1981).

Results

One hundred and sixty-one positive clones were identified from screenings based on colony or plaque hybridizations and were sequenced. Sixty-two (39%) contained repetitive $[GA]_n$ ($N = 35$) and $[CA]_n$ ($N = 27$) regions. A large number of microsatellite-containing clones ($N = 36$) were unusable because of the close proximity of repeat regions to the cloning site or were found to contain small repeat regions (repeats) or incomplete repeats (i.e., presence of single or multiple base pair substitutions within the repeat region). The high number of clones with *Sau*3AI restriction sites near repeat regions suggests that alternative restriction enzymes may be more appropriate for future libraries. Twenty-six positive clones containing microsatellite repeats had adequate flanking sequence to develop PCR primers (Table 1).

PCR amplification products of the expected size (based on the cloned sequence) were obtained for 22 of 26 microsatellite primer pairs tested, for at least one species (Table 2). Four primer pairs failed to produce unambiguous PCR products and were not used in subsequent analyses. While attempts were not made to optimize conditions for each species, most primer pairs amplified regions of the appropriate size in the majority of species tested using PCR conditions developed for chinook (loci *One*μ1–18) or sockeye (loci *One*μ19–22). Two primer pairs (*One*μ15 and *One*μ16) amplify what appears to be duplicated loci based on the presence of multiple alleles (3) of the appropriate size in sockeye and chinook salmon (data not shown). Locus *One*μ13 exhibited null alleles in high frequency in chinook salmon (see beyond).

Analyses of chinook salmon collected from each of three spawning aggregations from the Yukon River confirm the utility of these loci for population genetic analyses. Twelve of 16 loci were variable (mean $H_{DC} = 0.254$) with 2–11 alleles segregating at each variable locus within a population (Table 3). Many variable loci were allozyme-like in that a common allele was typically present in high frequency accompanied by one or few rare alleles. Several loci (e.g., *One*μ2, *One*μ8, and *One*μ14; Table 3) were highly polymorphic. Genotypic frequencies for each of 12 variable loci surveyed for the three populations conformed to Hardy–Weinberg expectations ($P > 0.05$) with the exception of locus *One*μ13, which was characterized by high frequencies of a null allele(s) (Table 3).

Populations differed greatly in allele frequency (Table 3). Heterogeneity in allele frequency among populations was documented for 7 of 12 polymorphic loci (mean $\theta = 0.036$, $P < 0.01$; Table 3).

The degree of interpopulation variation was further quantified using genetic distance measures (Fig. 1). Using a standard distance measure (Nei 1972), a measure based on the proportion of shared alleles (D_{AS}, Bowcock et al. 1994), and one based on population allele frequency and repeat copy scores (Δ, Goldstein et al. 1995), the Klondike and McQuesten populations were genetically most similar, while the Klondike and Stoney Creek populations were genetically most divergent (Fig. 1). Microsatellite results, in terms of the rank ordering of interpopulation estimates of divergence, and thus the topologies of resulting UPGMA phenograms, agreed with results from an earlier allozyme survey of these same populations (Wilmot et al. 1992; Fig. 1).

Discussion

Analyses using novel salmonid microsatellite loci revealed several major results. First, the potential of using heterologous PCR primers was explored and a majority of the microsatellites appear to be conserved across a diverse number of salmonid species. Second, the utility of these markers for population genetic analyses was confirmed. The majority of microsatellite loci surveyed were polymorphic, and many showed significant spatial heterogeneity in allele frequency among chinook salmon populations within one major river drainage. Finally, results of microsatellite population surveys agreed with those from an earlier allozyme study of the same populations, suggesting wide utility for a variety of basic and applied research questions.

Use of heterologous PCR primers and conservation of microsatellite loci

Presence of amplification products of expected size (based on sequences of sockeye clones) for each of 22 microsatellite

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Table 1. Characteristics of 22 sockeye salmon (*Oncorhynchus nerka*) microsatellite PCR primers, amplification conditions, repeat motifs, and expected product sizes.

Locus	Length (bp) of PCR product[a]	Repeat motif[b]	Annealing temp. (°C)[c]	Primer sequences (5′–3′)[d]
*One*μ1	113	$[GA]_{14}$	60–50	F: GTCTTACCAAATGTCTTCCTCCT
				R: GCCATTTAGCATACGATTTTATC
*One*μ2	188	$[GA]_{11}$	60–50	F: GGTGCCAAGGTTCAGTTTATGTT
				R: CAGGAATTTACAGGACCCAGGTT
*One*μ3	186	$[GA]_{18}$	52	F: TCTCCTTGGTCTCTCTGTCCCTT
				R: CTATCAGCCAATCGCATCAGGAC
*One*μ4	124	$[GA]_{23}$	60	F: TAATTTACATATCAGGTTCTGCC
				R: TATGCTAGTCATGGCTCTTACAT
*One*μ5[e]	178	$[CA]_{12}$	60	F: AACACACCAGCTGTGAAAACAAA
				R: TGTCTATCGCCAATCTCTCTGCT
*One*μ6	222	$[GA]_{18}$	60	F: CAGAGTGGCCTAGATGCTTTAAT
				R: CCACACACCAAATCCTACCCTTA
*One*μ7	190	$[GA]_{11}$ $N_2[GA]_4$	52	F: ACACTGCAAACACTCTGCTTACT
				R: CAAGAAGAAACCCTGTCCTCAAG
*One*μ8	208	$[CA]_{24}$	60	F: AACATTCTGGGATGACAGGGGTA
				R: CTGTTCTGCTCCAGTGAAGTGGA
*One*μ9	230	$[CA]_{54}$	60	F: CTCTCTTTGGCTCGGGGAATGTT
				R: GCATGTTCTGACAGCCTACAGCT
*One*μ10	133	$[CA]_{25}$	60–50	F: ATGGGGAACAGAAGAGGAAT
				R: CTGTAGGTGTGAAATGTATTTAAA
*One*μ11	152	$[CA]_{13}$	60	F: GTTTGGATGACTCAGATGGGACT
				R: TCTATCTTTCCTGTCAACTTCCA
*One*μ12	163	$[GA]_{24}$	60	F: ACTTATGCTAGTCATGGCTCTT
				R: TCGGTCATCGAAAGATACTTTT
*One*μ13	164	$[GA]_{20}$	52	F: TCATACCCCATGCCTCTTCTGTT
				R: GATGAGTGAAAGAGAGGGAGCGA
*One*μ14	143	$[CA]_{26}$	52	F: AGAAACATGAGAACAGTCTAGGT
				R: CCTTATGAGTTTGGTCTCCATGT
*One*μ15[f]	109	$[CA]_7$ $N_2[CA]_{13}$	52	F: AGCTTGACATCATAAAATGCGTC
				R: TTTCTTCTCTCATTCTCACACGA
*One*μ16[f]	142	$[CA]_{33}$	60	F: ATGCTGTAACCAGTGAATCCCTT
				R: TATCAACAGAATGCCAACCTTTT
*One*μ17	121	$[GA]_{20}$	60	F: ATGGCAGGATTGTTTTAGGTTGT
				R: GCCATGAGGAAGACACATCAATA
*One*μ18[e]	179	$[CA]_{16}$	54	F: ATGGCTGCATCTAATGGAGAGTAA
				R: AAACCACACACACTGTACGCCAA
*One*μ19	149	$[CA]_{33}$	56	F: CTGGAAAGCACAGAGAGAGCCTT
				R: TCCAACAGTCTAACAGTCTAACCA
*One*μ20	163	$[CA]_{16}$	60–50	F: TCTGTGGACAAAACATGAGATTA
				R: CTCCCATTTTCCCATTTATTGTT
*One*μ21	145	$[GA]_{35}$	56	F: GGTTACAGTGGGTTCACTCTACA
				R: GTTATGACAACAGTCTCTGTCGC
*One*μ22	168	$[GT]_{22}$	58	F: TTCTCTACAGGCGATGAACTGAT
				R: TTCTTACCTCCACGATGACACAA

[a]Expected PCR product size based on cloned sockeye salmon allele of specified repeat number. Product sizes may vary in other species.

[b]Core repeat motif from cloned sockeye salmon sequence.

[c]PCR annealing temperatures were optimized either for chinook (loci *One*μ1–18) or sockeye salmon (loci *One*μ19–22). Optimal conditions may vary for additional species (see Table 2). Ranges in temperature refer to "touch-down" conditions (annealing temperature are decreased by 2°C at 2-cycle intervals and 30 cycles are run at the final temperature).

[d]F and R refer to forward and reverse primer sequences, respectively.

[e]Two distinct genomic regions amplified. The lower molecular weight fragment is of the expected size.

[f]Multiple allelic banding patterns observed for sockeye and chinook salmon suggest gene duplication.

primer pairs suggests that dinucleotide repeat regions and sequences flanking these motifs are highly conserved across species differing greatly in degree of relatedness (Table 2). The degree of homology has some degree of phylogenetic basis in that a greater number of primer pairs produce amplification products within the genus *Oncorhynchus* compared with those of more distantly related (Stearley and Smith 1994) taxa. Findings of high interspecific homology are consistent with results

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Table 2. Cross-species amplification using 22 pairs of sockeye salmon (*Oncorhynchus nerka*) microsatellite primers.

	Oncorhynchus							*Salmo*	*Thymallus*	*Salvelinus*
Locus	*kisutch*	*gorbuscha*	*keta*	*nerka*	*tshawytscha*	*mykiss*	*clarki*	*salar*	*arcticus*	*malma*
*One*μ1	*	+	+[a]	+	+	+	+	+	*	+
*One*μ2	+	*	+[a]	+	+	+	+	+	+	*
*One*μ3	*	+	*	*	+	*	+	+	*	*
*One*μ4	+	+	*	+	+	+	*	+[a]	*	*
*One*μ5	*	*	+[a]	+	+	+	+	+	*	*
*One*μ6	+	+	+	+	+	+	+	+	+	*
*One*μ7	+	*	+	+	+	+	+	*	+	+
*One*μ8	–	+[a]	*	+	+	+	+	–	+	*
*One*μ9	*	+	+	+	+	*	*	+	+	*
*One*μ10	*	+	+	+	+	+	+	+	+	+
*One*μ11	*	*	+	+	+	+	+	+	*	*
*One*μ12	*	*	–	+	+	*	*	–	*	*
*One*μ13	*	+	+	+	+	+	*	+	*	*
*One*μ14	*	*	+[a]	+	+	*	+	+	*	*
*One*μ15[b]	*	*	*[a]	+	+	+	+	+	*	+
*One*μ16[b]	*	*	–	+	*	–	*	–	*	–
*One*μ17	+	+	+	+	+	+	+	+	+	+
*One*μ18	+	*[a]	+	+	+[c]	+	+	+[c]	*	*
*One*μ19	+[a]	+[a]	+	+	+	+	+	–	*	–
*One*μ20	*	+	*	+	*	+	*	*	*	*
*One*μ21	*	*	*	+	–	*	*	–	*	*
*One*μ22	*	*	*	+	+	–	*	–	+	–

Note: Symbols associated with each locus-species category refer to the presence or absence and quality of PCR products based on conditions optimized for chinook salmon: +, a single clear PCR product was obtained; *, a PCR product of the expected size was obtained but conditions were suboptimal; –, no PCR product was observed.

[a]PCR product was not of the size range (>100 bp difference) documented for the cloned sockeye salmon sequence.

[b]Multiple PCR products.

[c]Duplicated locus.

from other vertebrates. Schlotterer et al. (1991) found that homologous loci could be amplified from a diverse range of toothed (Odontoceti) and baleen (Mysticeti) whales with estimated divergence times of 35–40 Myr. Moore et al. (1991) found microsatellites were conserved across species as diverse as primates, artiodactyls, and rodents. Microsatellites isolated in domestic dogs were used in studies of a variety of canid species (Gotelli et al. 1994; Roy et al. 1994). Use of heterologous PCR primers should significantly reduce the cost of developing similar markers for other salmonid species.

Spatial heterogeneity in allele frequency among populations

Allelic variation within populations was high, both in terms of the number of alleles per locus and single- and multi-locus heterozygosity (Table 3). Heterozygosities were substantially greater than earlier estimates based on allozyme analyses (H_E = 0.201–0.226 versus 0.080–0.098, respectively). Relatively low sample sizes preclude any definitive interpretation of the utility of the presence of rare alleles within specific populations (i.e., in the context of estimating gene flow; Slatkin 1985). However, various criteria were proposed for determining appropriate sample size requirements for various statistical analyses (Chakraborty 1992).

The majority of polymorphic microsatellite loci surveyed exhibited significant spatial variation in allele frequency across the three sampled chinook populations (Table 3). The topologies of each UPGMA phenogram, in terms of identifying the rank-order of degree of between-population variation, were the same for each genetic distance measure used (Fig. 1). Estimates of Nei's (1972) interpopulation genetic distances were concordant between microsatellite and earlier allozyme analyses for these populations. Similar findings were reported for comparisons of minisatellite, microsatellite, and allozyme data sets collected for *Bufo bufo* (Scribner et al. 1994), suggesting that different loci classes are able to capture the same signature of microevolutionary events. Differences in branch length were observed, presumably because of the unique properties of each of the three distance measures employed (see below).

Comparison of the utility of microsatellites and other nuclear genetic markers

The utility of any genetic marker for ecological or evolutionary genetic studies depends principally on the research question of interest and the inherent properties of markers themselves. Studies at the intraspecific level routinely rely on protein electrophoresis and mtDNA. Indeed, for years allozyme electrophoresis has been the workhorse of empirical fisheries population genetics. However, for various reasons (e.g., low levels of detectable polymorphism, sample storage requirements, desirability of nondestructive sampling, and possible confounding effects of selection), allozymes may not always be appropriate for population-level analyses. Microsatellites are assayed using PCR-based techniques and thus are amenable to nondestructive sampling (i.e., using fin clips,

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Table 3. Allele frequencies and estimates of genetic variability for 16 microsatellite loci surveyed for three spawning populations of Chinook salmon (*Oncorhynchus tshawytscha*) from the Yukon River, Yukon Territories, Canada.

Locus	Allele (bp)	McQuesten ($N = 29$)	Klondike ($N = 27$)	Stoney Creek ($N = 27$)	θ^a
*One*μ1	142	1.000	1.000	1.000	
*One*μ2	194	0.707	0.685	0.796	0.002
	196	0.103	0.148	0.037	
	200	0.138	0.111	0.074	
	204	0.017	0.000	0.000	
	208	0.000	0.037	0.056	
	210	0.000	0.000	0.037	
	216	0.034	0.019	0.000	
*One*μ3	162	0.034	0.037	0.185	0.078*
	166	0.966	0.963	0.815	
*One*μ4	95	0.948	0.778	0.963	0.089**
	97	0.000	0.037	0.000	
	109	0.017	0.185	0.019	
	111	0.034	0.000	0.019	
*One*μ5	183	1.000	1.000	0.923	0.063
	185	0.000	0.000	0.077	
*One*μ6	206	1.000	1.000	1.000	
*One*μ7	191	0.121	0.074	0.130	0.001
	193	0.879	0.926	0.870	
*One*μ8	156	0.241	0.241	0.167	0.013
	158	0.000	0.019	0.000	
	160	0.000	0.074	0.037	
	170	0.537	0.370	0.593	
	174	0.056	0.167	0.056	
	178	0.148	0.130	0.148	
	184	0.019	0.000	0.000	
*One*μ9	165	0.000	0.000	0.148	0.137**
	169	1.000	1.000	0.852	
*One*μ10	135	0.103	0.222	0.019	0.074**
	137	0.621	0.611	0.463	
	141	0.000	0.019	0.000	
	143	0.276	0.148	0.519	
*One*μ11	139	1.000	1.000	1.000	
*One*μ12	131	0.865	0.759	0.963	0.057**
	133	0.000	0.037	0.000	
	145	0.135	0.204	0.037	
*One*μ13[b]	148	0.048	0.000	0.000	Not tested
	150	0.167	0.000	0.000	
	152	0.143	0.438	0.022	
	154	0.524	0.250	0.522	
	174	0.119	0.313	0.457	
	null	0.525	0.678	0.385	
*One*μ14	187	0.000	0.019	0.019	0.017*
	189	0.034	0.000	0.000	
	191	0.017	0.000	0.000	
	193	0.103	0.074	0.093	
	195	0.000	0.000	0.019	
	197	0.121	0.130	0.074	
	199	0.000	0.000	0.093	
	201	0.069	0.222	0.204	
	203	0.397	0.296	0.296	
	209	0.155	0.111	0.000	
	213	0.000	0.019	0.000	
	215	0.069	0.111	0.000	
	237	0.017	0.000	0.000	
	239	0.000	0.000	0.037	
	241	0.000	0.000	0.056	
	243	0.017	0.000	0.056	
	245	0.000	0.000	0.037	
	247	0.000	0.019	0.000	
	253	0.000	0.000	0.019	
*One*μ17	96	1.000	1.000	1.000	
*One*μ18	167	0.792	0.827	0.600	0.051*
	177	0.146	0.077	0.175	
	179	0.063	0.096	0.225	
Mean±SE					0.036±0.012
Observed heterozygosity (direct count)		0.201	0.226	0.221	
Expected heterozygosity (under Hardy–Weinberg)		0.227	0.267	0.239	
Pc[c]		0.615	0.615	0.769	
A[d]		2.5	2.5	2.5	

[a] Significance of θ: *, $P < 0.05$; **, $P < 0.01$.

[b] Locus not used in calculations of θ and genetic distance (Fig. 1) because of the presence of null alleles.

[c] Percentage of loci polymorphic.

[d] Mean number of alleles per locus.

scales, blood) because of the lack of constraints on amounts of DNA needed. Microgram quantities of DNA are routinely obtained from adapose or dorsal fin clips, and all loci reported herein were successfully assayed using these material sources. This is of particular concern when sampling from protected parks and preserves or when sampling threatened and endangered species. Because of the tissue-specific expression of many proteins, large-scale allozyme analyses frequently require destructive sampling. Field collections of samples for DNA-based analyses does not require cryopreservation. In addition, microsatellites occur in noncoding regions and are thought to be free from selective constraints, an assumption often accepted but rarely tested for allozyme variants (see Verspoor and Jordon 1989; Karl and Avise 1992).

Microsatellites also have distinct advantages over other classes of highly polymorphic VNTR loci such as minisatellites. Minisatellite variation is routinely assessed using Southern-blotting techniques. Measurement errors within and across gels may exceed repeat unit length. Thus, estimates of fragment mobilities may not correspond directly to repeat copy number. The continuous distribution of fragment sizes frequently necessitates allelic classifications based on "floating" (Weir 1992) or "fixed" (Budowle et al. 1991) bins. Both the precision of allelic classification (based on bin assignment) and the abundance of allelic and genotypic classes raises a challenge for testing classical population genetics models (Scribner et al. 1994). Conversely, microsatellite allelic designation can be assigned unambiguously, as PCR products can

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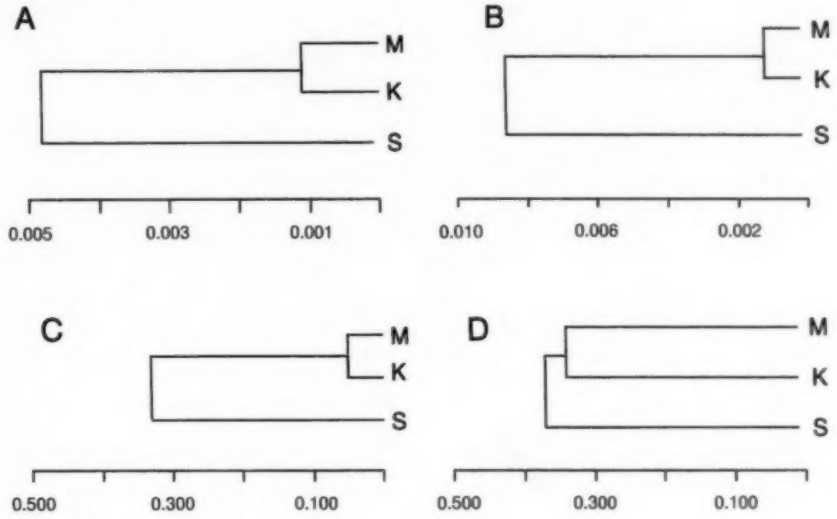
Fig. 1. UPGMA phenograms derived using three measures of genetic distance, summarizing relationships among three populations of chinook salmon (*O. tshawytscha*) from the Yukon River. M, McQuesten; K, Klondike; S, Stoney Creek. (A) *D* (Nei 1972) for allozymes. (B) *D* (Nei 1972) for microsatellites. (C) Δ (Goldstein et al. 1995) for microsatellites. (D) D_{AS} (Bowcock et al. 1994) for microsatellites.



be run on high resolution polyacrylamide sequencing gels and scored using a reference sequence standard (e.g., M13 or the cloned allele). The ability to precisely score alleles greatly enhances the potential for comparisons across studies if locus nomenclature (see Tautz 1993) and allelic standards are established with the rigor pursued by researchers employing protein allozymes.

Unique properties of microsatellite loci

The high information content relative to more standard genetic markers suggests great utility to a variety of fisheries research and management issues. The question is how to make the best use of the unique properties (i.e., rate and mode of mutation) of these loci. What assumptions must be satisfied when conducting statistical analyses?

The role of mutation has traditionally been downplayed as an evolutionary force impacting the genetic divergence among populations. If mutation rates are much less than migration rates, than for neutral alleles, divergence (as estimated by F_{st}) is shown to be nearly independent of mutation rate (Crow and Aoki 1984). If the mutation rate is high relative to the rate of interpopulation gene flow, then for neutral and highly mutable loci, novel mutations might contribute significantly to estimates of interpopulation variation (Scribner et al. 1994).

High mutation rates and the reported stepwise mutation process by which alleles mutate to smaller or larger size (i.e., by small number of repeat units; Schlotterer and Tautz 1993; Weber and Wong 1993) does not erase information about ancestral states. Evidence from direct studies of mutations in human families (e.g., Weber and Wong 1993) revealed that the size of a new mutant allele depends on the size of the allele that mutated (i.e., nearly all mutations differ from their ancestors by one or two repeat units). Regardless of whether all mutations are of only small incremental changes as assumed under the stepwise mutation models of Valdes et al. (1993), Shriver et al. (1993), and Goldstein et al. (1995), or are occasionally of larger magnitude as suggested under the two-step mutation model of Di Rienzo et al. (1994), assumptions made when using numerous standard measures of divergence (i.e., measures based on divergence in allele frequency such as F_{st}; Wright 1951) to estimate N_m or other demographic parameters may not be satisfied (Slatkin 1995). High mutation rates could also result in convergence in allele size (i.e., identify in state) in different geographic locales independent of common ancestry. Additional complications may arise because of the presence of nonamplifying or null alleles that are due to mutations in one of the priming sites (Callen et al. 1993; Pemberton et al. 1995).

Determining the relative importance of various evolutionary forces that cumulatively affect the degree of interpopulation divergence in microsatellite allele frequencies (i.e., drift, migration, mutation) will require further empirical and theoretical work. Likewise, the assumptions and caveats underlying the use of various measures of interpopulation differentiation will certainly be refined with the accumulation of additional empirical data. Recent attempts were made to provide population statistics appropriate for microsatellite analyses (Bowcock et al. 1994; Goldstein et al. 1995; Slatkin 1995). Goldstein et al. (1995) derived a distance measure (Δ) that is reported to be linear with time when used for loci undergoing a strict stepwise mutation process. Unlike other distance measures (e.g., Nei's distance; Nei 1972) that measure interpopulation differentiation caused by sampling, Δ is a function of mutation rate and not population size. Using simulation studies, these authors show that for populations separated for 100 generations, divergence (Δ) is linear over time.

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Bowcock et al. (1994) defined a distance measure between pairs of individuals ($1-P_S$), where P_S is the number of shared alleles, summed over loci, divided by twice the number of loci assayed. This measure can be extended to populations in a manner similar to that used for band-sharing coefficients in multilocus minisatellite analyses.

Goldstein et al. (1995) showed that the estimator Δ and distance measures based on the proportion of shared alleles (Bowcock et al. 1994) may be useful in identification of group membership of individuals within admixed groups composed of a set of previously distinct populations. If sufficient differences exit across subpopulations, it is possible in principle to assign individuals within an admixed group to their population of origin using clustering programs.

Interpopulation divergence, as quantified using various distance measures, increases monotonically (though in a nonlinear fashion) as a function of the time since separation. Over relatively short periods of time, drift would be expected to be the most pervasive evolutionary force causing divergence in allele frequencies. Therefore, genetic distance measures that are based on differences in allele frequency (e.g., Nei's distance) would be expected to track microevolutionary changes more adequately than measures that incorporate mutational processes (e.g., Δ). Mutation-based distance measures may prove more appropriate for resolving deeper branches of a population phylogeny, extending over greater periods of post-separation time.

Differences among distance measures are exemplified in Fig. 1 by variation in interpopulation branch lengths. While all measures show the Klondike and McQuesten populations to be more closely related than either are to Stoney Creek (a result consistent with population geographic proximities), the branch lengths based on Δ (Fig. 1C) are a order of magnitude larger than those based on Nei's genetic distance (Figs. 1A and 1B). This is presumably a function of the wide range of allele sizes (and frequency heterogeneity) at some loci (i.e., *One*μ14). Resolution of the Klondike–McQuesten versus Stoney Creek branches was less clear based on the proportion of shared alleles (D_{AS}; Fig. 1D), though the results, in part due to high number of private alleles, were consistent with topologies derived from other measures.

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Relations between trophic state indicators and fish in Florida (U.S.A.) lakes[1]

Roger W. Bachmann, Bradley L. Jones, Donald D. Fox, Mark Hoyer, Lawrence A. Bull, and Daniel E. Canfield, Jr.

Abstract: Total fish biomass per unit area was positively correlated with total phosphorus, total nitrogen, chlorophyll *a*, and inversely correlated with Secchi disk transparency in 65 Florida (U.S.A.) lakes selected to range from oligotrophic to hypereutrophic. Species numbers were positively related to lake surface area but not trophic state. There were some shifts in species composition with changes in trophic state, though only a few species showed significant changes in their standing crops. In particular the recreationally important centrarchids did not show important changes with trophic state, and there were no critical points on the trophic spectrum where there were dramatic changes in fish abundance or standing crops. The facts that Florida lakes do not have deep, cold hypolimnia, do not have salmonid species, and have no ice in the winter are among the possible reasons that the more eutrophic Florida lakes do not show the same changes in fish populations often described for northern lakes.

Résumé : Il y avait une corrélation positive entre la biomasse totale de poissons par unité de surface et le phosphore total, l'azote total et la chlorophylle *a*, et une corrélation inverse entre cette biomasse et la transparence mesurée à l'aide du disque de Secchi, dans 65 lacs de Floride (É.-U.) allant d'oligotrophes à hypereutrophes. Les nombres d'espèces étaient liés positivement à la superficie des lacs, mais non à leur état trophique. La composition des espèces variait quelque peu avec l'état trophique, mais seules quelques espèces accusaient des différences importantes dans le nombre d'individus. Plus particulièrement, les centrarchidés importants sur le plan récréatif n'accusaient pas d'importants changements avec l'état trophique, et le spectre trophique ne présentait aucun point critique correspondant à des changements dramatiques de l'abondance ou du nombre d'individus. L'absence d'un hypolimnion froid et profond, l'absence de salmonidés et l'absence de glace en hiver comptent parmi les raisons pouvant expliquer pourquoi les lacs de Floride plus eutrophes n'accusent pas les mêmes variations de population de poissons dont on a souvent fait état dans les lacs nordiques.
[Traduit par la Rédaction]

Introduction

Eutrophication is a concern in Florida where a growing human population and changes in land use are increasing nutrient inputs to many lakes. The impacts of excess algal populations on water transparency and the general appearance of Florida lakes are well known (Canfield and Hodgson 1983; Canfield et al. 1985); however, less information is available on the effects that changes in trophic state have on fish populations. There is special interest in Lake Okeechobee, a large (1730 km^2) and shallow (mean depth = 2.7 m) lake in south Florida where total phosphorus concentrations in the lake have increased over the past 20 years (Janus et al. 1990; James et al 1995). As Lake Okeechobee has a major recreational fishery, there is interest in knowing the potential impact of trophic state changes on fish populations in this and other Florida lakes.

There are many quantitative studies that support the expectation that an increase in productivity at the base of food chains in a lake should translate into an increase in the abundance of fish at higher trophic levels. Oglesby (1977) found high correlations between fish yields in lakes and summer phytoplankton standing crops as measured by chlorophyll *a* and also between fish yield and annual primary productivity. Melack (1976) studied tropical lakes in Africa and India and found that fish yields increased logarithmically as primary production increased arithmetically. McConnell et al. (1977) found that fish yield and gross photosynthesis were highly correlated in six experimental ecosystems involving a variety of fishes. Yurk and Ney (1989) found a strong correlation between fish standing crops and total phosphorus in 22 southern Appalachian reservoirs. Downing et al. (1990) found that fish community production was correlated with annual phytoplankton production, mean total phosphorus concentrations, and annual average fish standing stock. Hanson and Leggett (1982) found that both fish biomass and yield were predicted by lake total phosphorus concentrations as well as by the ratio of macrobenthos biomass to lake mean depth. Lee et al (1991) reanalyzed the data of Oglesby (1977) and Hanson and Leggett (1982) and found a strong correlation between fish yield and normalized phosphorus loading to lakes. In Florida, Kautz (1980) found that total fish biomass increases with trophic state as measured

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R.W. Bachmann,[2] M. Hoyer, and D.E. Canfield, Jr. Department of Fisheries and Aquatic Sciences, University of Florida, Gainesville, FL 32653, U.S.A.
B.L. Jones. South Florida Water Management District, P.O. Box 24680, West Palm Beach, FL 33416, U.S.A.
D.D. Fox and L.A. Bull. Florida Game and Fresh Water Fish Commission, 3991 SE 27th Court, Okeechobee, FL 34974, U.S.A.

[1] Journal Series No. R–04754 of the Florida Agricultural Experiment Station, Gainesville, Fla.
[2] Author to whom all correspondence should be addressed. e-mail: rbach@nervm.nerdc.ufl.edu

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Fig. 1. Location of lakes sampled for this study.

by total nitrogen concentrations. Bays and Crisman (1983) found a similar relationship with algal chlorophylls. The overwhelming evidence from these and other studies is that, as lakes become more eutrophic, the standing crops, productivity, and yields of fish increase. Ryding and Rast (1989) cite this as one of the positive properties of eutrophication in relation to the production of fish for food. In addition to the studies based on standing crops or commercial fish yields, Jones and Hoyer (1982) found that the yields of sport fish to angler harvest in 26 lakes and reservoirs in Iowa and Missouri were highly correlated to mean summer phytoplankton standing crop as measured by chlorophyll *a*.

Conversely, there are many observations in the literature that suggest that, as eutrophication proceeds, there is a decline in the quality of a fishery in terms of species composition. Larkin and Northcote (1969) reviewed a number of studies showing changes in fish populations as a result of eutrophication. These included changes in the fish fauna of Lake Erie coincident with eutrophication, the loss of *Coregonus* from Lake Muritz in northern Germany, and the loss of whitefish in the Bodensee. In a recent review of the literature on eutrophication and fisheries, Lee et al (1991) agree that eutrophication can significantly affect the types of fish in a water body. They cite various studies showing a decline in species richness in response to an increase in trophic state. Persson et al. (1988) showed that, in going from the least productive to the most productive in a series of Swedish lakes, there was a major decrease in the proportion of piscivorous fish. In his study of 22 lakes in Florida, Kautz (1980) concluded that sport fishes reach maximum biomass and optimum densities in mesotrophic–eutrophic lakes but suffer adverse effects with further enrichment.

Many of these previous studies have been carried out on deep, northern lakes with different fish communities from those in Florida. The results may not directly apply to shallower lakes in a warmer climate. The purpose of our study was to examine data on trophic indicators and fish populations from a large sample of Florida lakes to determine the relationships between lake trophic state and the total biomass, species richness, species composition, and standing crops of fish.

Methods

Fish sampling

Most of the data were collected on 60 Florida lakes (Fig. 1) that were sampled specifically to develop empirical relationships between fish and trophic state variables (Canfield and Hoyer 1992). Ten to 17 lakes were sampled each year between June 1986 and June 1990. The lakes encompassed a range of trophic states and included lakes within the major trophic categories (oligotrophic, mesotrophic, eutrophic, and hypereutrophic) following the guidelines of Forsberg and Ryding (1980). Rotenone sampling for fish was conducted once during the warm season (May–November) at each lake to determine standing crop and community structure. Two to twelve 0.08-ha blocknets were set at each lake depending on lake size. The majority of lakes were sampled with six nets, but Little Fish Pond (surface area 2 ha) was sampled with only 2 nets while Lake Apopka (surface area >12 000 ha) was sampled with 12 nets. Equal numbers of blocknets were set in littoral (with one side being the shore) and limnetic habitats. Blocknet sampling followed procedures outlined by Shireman et al. (1983). Biomass (kilograms per hectare) estimates were calculated for each fish species in each net and weighted by habitat (littoral and open-water area) to obtain whole-lake estimates.

Each lake was also sampled by gillnets and electrofishing to provide additional information on fish species composition. Three experimental gillnets were fished in the majority of lakes, but six nets were set in Lake Apopka. Nets were fished along the bottom in water depths >2 m once for 24 h during the summer sampling period. Gillnets were 50 × 2.4 m and each gillnet had five 10-m panels of different mesh size (bar mesh sizes: 19, 25, 38, 51, and 76 mm). Electrofishing was conducted at each lake once during the warm season sampling period in nearshore areas in all habitats. Two to 10 electrofishing samples (10 min transects) were taken at each lake depending on lake size. Six samples were taken at most of the lakes, but only two samples were taken at Little Fish Pond (lake surface area 2 ha). Ten samples were taken from Lake Harris (lake surface area 5580 ha). Electrofishing transects were evenly spaced around each lake and electrofishing was done for 10 min with continuous current.

For Lake Okeechobee, data were obtained from fish population surveys made by the Florida Game and Fresh Water Fish Commission (Fox et al. 1992). Fish assemblages were sampled for biomass in five vegetation communities in the northern, southern, and western regions of the lake. Sampling was carried out in the fall seasons of 1989, 1990, and 1991. Three blocknets, 3.4 m deep with 3-mm bar mesh and encompassing 0.08 ha, were set in each vegetation community in each sample area during each year. Three Wegner rings (4.0 m^2) (Wegner et al. 1973) were randomly placed within each blocknet to estimate abundance of fish less than 6 cm total length (Miller et al. 1990). Emulsified rotenone (5%) was applied within the net at a concentration of 2 $mg \cdot L^{-1}$, and fish were picked up for 3 days. Percentages of fish collected on the second and third day in similar vegetation types were used to adjust numbers of fish collected in Wegner rings removed after the first day. Collected fish were sorted by species, divided into 2-cm size-clases (i.e., 4-cm group, 4.00–5.99 cm), enumerated, and weighed to the nearest gram. Second- and third-day weights were assigned from length–weight tables derived from first-day weights.

Although no fish biomass samples were taken from the open-water areas of Lake Okeechobee, trawl sampling data were used to check the fish species list (Fox et al. 1992). A 4.88-m, semiballoon trawl net having 35-mm stretched mesh in the wings and 25-mm stretched mesh in the bag was fished in July and January of each year beginning

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Table 1. Total number of fish species, whole lake fish standing crop, littoral zone fish standing crop, total phosphorus (TP), total nitrogen (TN), chlorophyll *a* (Chl *a*), Secchi depths (SD), mean depths (*Z*), and surface areas (SA) for 65 Florida lakes.

Lake	No. of fish species	Standing crop: Whole lake ($kg \cdot ha^{-1}$)	Standing crop: Littoral zone ($kg \cdot ha^{-1}$)	TP ($\mu g \cdot L^{-1}$)	TN ($\mu g \cdot L^{-1}$)	Chl *a* ($\mu g \cdot L^{-1}$)	SD (m)	*Z* (m)	SA (ha)
Okeechobee	41		372	92	1480	30	0.5	2.7	173 000
Crescent	44		284	30	1100	34	0.6		6 290
George	42		379	56	1400	79	0.7		18 600
Istokpoga	31		219	210	700	10	0.9	1.8	11 207
Tohopekaliga	34		507	138	1535	44	0.8	2.0	9 194
Alligator	18	112	211	371	2367	84	0.5	1.1	137
Apopka	24	24	81	140	3789	127	0.3	1.6	12 412
Baldwin	19	65	361	21	530	18	1.6	4.5	80
Barco	9	7	11	2	82	1	5.4	4.4	13
Bell	16	44	63	17	641	20	1.5	2.7	32
Bivens Arm	12	683	326	384	3256	241	0.4	1.2	76
Bonny	16	79	420	59	1858	40	0.6	2.0	143
Brim Pond	6	103	80	9	624	8	2.2	4.0	3
Bull Pond	22	69	117	11	522	3	1.4	2.3	11
Carlton	25	308	749	92	3228	173	0.4	3.6	155
Carr	15	207	8	19	874	11	1.8	1.9	254
Catherine	17	103	91	2	303	2	3.2	3.2	41
Clay	7	136	85	7	356	4	4.0	2.3	5
Clear	12	41	227	21	761	21	1.3	5.9	64
Conine	20	179	174	1043	2056	110	0.5	3.5	96
Crooked	12	106	113	7	313	5	3.1	2.3	8
Cue	6	10	44	5	91	2	5.8	3.5	59
Deep	17	88	89	2	158	1	5.1	3.0	4
Douglas	17	81	81	11	1122	2	1.5	1.2	16
Fish	21	129	210	25	935	18	1.0	1.9	89
Gate Lake	14	69	82	28	407	20	1.1	1.8	8
Grasshopper	21	135	161	6	259	1	3.7	2.7	59
Harris	34	109	206	28	1550	37	0.6	4.0	5 580
Hartridge	21	47	44	11	485	4	2.3	3.4	176
Holden	16	84	258	44	1226	64	0.5	4.5	102
Hollingsworth	16	150	1046	113	2517	135	0.3	1.5	144
Hunter	18	414	444	98	1723	82	0.5	1.7	340
Keys Pond	10	27	41	2	208	1	5.3	2.9	5
Killarny	18	230	403	21	603	22	1.0	4.7	96
Koon	23	46	45	5	687	3	1.4	1.5	44
Lawbreaker	4	32	42	1	108	1	5.5	4.3	5
Lindsey	13	78	167	19	636	6	1.9	2.2	55
Little Fish	8	240	251	21	1161	13	1.4	1.2	2
Live Oak	23	27	81	13	389	9	2.6	3.0	152
Lochloosa	29	149	160	32	1053	22	1.0	1.8	2 309
Loften	10	56	57	5	633	2	2.5	2.6	5
Marianna	21	193	244	26	1054	21	1.3	3.8	204
Mill Dam	29	42	36	11	462	4	2.7	5.7	85
Miona	24	63	62	12	867	8	1.5	2.3	169
Moore	15	42	96	5	353	3	5.3	2.9	28
Mountain	14	135	185	37	813	10	1.7	1.6	51
Mountain 2	17	47	193	17	331	2	2.4	3.3	55
Okahumpka	25	95	68	21	1033	11	1.4	0.9	271
Orienta	17	190	232	25	448	9	2.2	3.4	52
Pasadena	16	115	111	15	702	3	2.2	3.1	151
Patrick	25	170	173	10	1808	5	2.0	1.8	159
Pearl	20	84	111	28	819	228	0.9	2.0	24
Picnic	11	48	51	8	137	1	2.6	3.3	18
Round Pond	9	135	126	3	444	3	2.6	1.3	4

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Table 1 (*concluded*).

Lake	No. of fish species	Standing crop: Whole lake (kg·ha^{-1})	Standing crop: Littoral zone (kg·ha^{-1})	TP (μg·L^{-1})	TN (μg·L^{-1})	Chl *a* (μg·L^{-1})	SD (m)	Z (m)	SA (ha)
Rowell	30	463	615	66	910	47	0.8	1.3	147
Suggs	20	19	12	66	1249	4	0.5	2.0	73
Susannah	19	217	470	23	674	25	1.5	3.9	31
Swim Pond	15	60	61	25	1025	11	0.6	0.6	9
Thomas	13	98	147	22	759	10	1.8	3.9	55
Tomahawk	13	84	99	6	192	1	4.2	4.4	15
Turkey Pen	10	52	71	2	132	1	3.2	5.0	6
Wales	15	51	42	27	899	42	0.8	3.4	132
Watertown	18	147	290	27	777	24	1.0	3.8	19
Wauberg	22	674	464	166	1478	102	0.6	3.6	100
West Moody	18	170	220	14	584	2	2.8	3.5	39
Mean	18	131	200	60	949	32	2	3	3 733
Median	17	92	147	21	759	10	1.45	2.7	59
Maximum	41	683	1046	1043	3789	241	5.8	5.9	173 000
Minimum	4	7	8	1	82	1	0.3	0.6	1.8

Note: For lakes Okeechobee, Crescent, George, Istokpoga, and Tohopekaliga the water chemistry is based on open-water samples and for the other lakes it is an average of open-water and littoral zone samples. Sampling was done between 1986 and 1992.

Table 2. Slopes and intercepts for regression equations and Pearson's correlations (r^2) between the trophic state variables total phosphorus (TP; mg·m^{-3}), total nitrogen (TN; mg·m^{-3}), chlorophyll *a* (Chl *a*; mg·m^{-3}), and Secchi disk depth (SD; m) in 65 Florida lakes.

Y-variable	*X*-variable	Slope	Intercept	r^2
Log_{10} Chl *a*	Log_{10} TP	0.887	–0.174	0.73
Log_{10} Chl *a*	Log_{10} TN	1.405	–2.985	0.69
Log_{10} TN	Log_{10} TP	0.498	2.180	0.66
Log_{10} SD	Log_{10} Chl *a*	–0.466	0.617	0.78
Log_{10} SD	Log_{10} TP	–0.473	0.779	0.75
Log_{10} SD	Log_{10} TN	–0.790	2.394	0.78

in July 1987 and continuing through January 1991. In July 1987, one 10-min haul was taken at each of 25 open-water sites. In all subsequent sampling periods, two 10-min hauls were made at 27 sites, although some stations were missed during low-water periods in July 1990.

To add more large lakes to the data set, we also used fish biomass data (Eisenhauer et al. 1993; Moxley et al. 1993; Moyer et al. 1992, 1993) collected by the Florida Game and Fresh Water Fish Commission in the littoral areas of lakes Crescent, George, Istokpoga, and Tohopekaliga (Fig. 1). In the fall of both 1990 and 1991, eight littoral, standard rotenone blocknet samples (0.1 ha) were taken from Lake Crescent and four from Lake George. Data for species composition were also obtained from two fall 0.4-ha limnetic blocknet samples taken in both years on Lake George. Fish biomass data from Lake Istokpoga and Lake Tohopekaliga were obtained from six (0.4 ha) littoral rotenone blocknet sites taken from August 1988 through August 1991 and fall 1990, 1991, and 1992, respectively. Additional information on species composition was obtained by electrofishing, gill nets, and Wegner rings.

We examined the potential effect of increasing lake trophy on species richness, defined as the number of different species of fish collected in each lake. First, we examined the cumulative number of fish species encountered in each lake as fishing effort increased to assess the likelihood that more sampling would add additional species. For the 60 lakes sampled by Canfield and Hoyer (1992), we constructed curves showing the cumulative numbers of fish species obtained as progressively more blocknets were fished. For each species in each lake, we used our catch records to determine the number of nets in which the species was found out of the total number of nets set. We then determined the probability that the species would be encountered for the first time in the first, second, third, and other nets set following the basic laws of probability (Dietrich and Kearns 1986). The expected frequencies for each species for each net were then summed to derive a curve of species found versus sampling effort. In each case the greatest increases in numbers of species found are in the first few nets with relatively little increase in the number of species caught as the last net is fished. As a measure of the flatness of the right-hand portion of the curve, the numbers of species seen after the next to last net was fished were expressed as a percentage of the number of species found after the last net was examined. In 41 of the 60 lakes, 95–100% of the species found in all nets were found by the next-to-last net. In only three cases was the value less than 90%. However, in addition to the species found in these blocknets, fish were also collected by gillnets and electroshocking to search for species that may have been missed.

For the five lakes sampled by the Florida Game and Freshwater Fish Commission, we compiled the cumulative numbers of species collected by all means for each year of sampling. For lakes Okeechobee, Istokpoga, and Tohopekaliga, 40 of 41, 30 of 31, and 32 of 34, respectively, of the species found were noted before the last year of sampling. Lakes Crescent and George were only sampled in 2 years with 36 of 44 and 38 of 42 of the species being found in the first year. We might expect to find a few more species in the latter two lakes with more sampling. It is noted that several marine species were found in these two lakes as a result of their close connection with the St. Johns River estuary and that they had more fish species than any of the other lakes in our study.

To assess whether trophic state played a role in determining the presence or absence of a fish species in lakes of a particular trophic state, we divided our lakes into four groups based on chlorophyll *a* concentrations in the water following the guidelines of Forsberg and Ryding (1980). Oligotrophic lakes had less than 3 mg·m^{-3} of chlorophyll *a* (n = 14); mesotrophic, 3–7 mg·m^{-3} (n = 12); eutrophic, 7–40 mg·m^{-3} (n = 26); and hypereutrophic, >40 mg·m^{-3} (n = 13). A chi-square test was used to determine if trophic state played a significant role in determining fish distribution in lakes. Many of the fish were found in fewer than 20 lakes, making the sample size too small for a valid chi-square test. Thus, we further grouped the lakes

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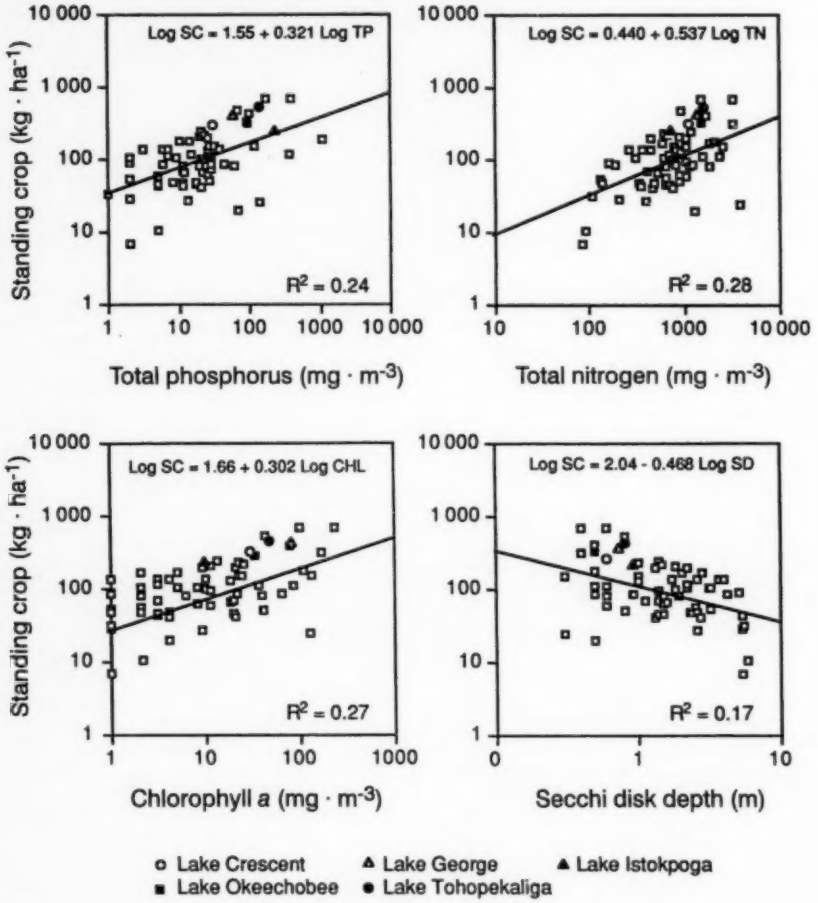
Fig. 2. Fish standing crops (kg·ha^{-1}) in relation to total phosphorus, total nitrogen, chlorophyll *a*, and Secchi disk transparency for 65 Florida lakes. The regression lines and r^2 values are based on the 60 lakes with whole-lake estimates of standing crops. The points for the five large lakes represent littoral fish standing crops only and are included for comparative purposes.



into oligotrophic–mesotrophic lakes and eutrophic–hypereutrophic lakes and tested to see if the frequencies of presence or absence were randomly distributed between the two groups. If any of the expected frequencies were less than five, we used the Fisher exact probability test (Siegel 1956) to test for significant differences in distributions.

To determine the effects of lake trophic state on the standing crops of individual fish species in the 60 lakes with weighted averages for fish standing crops, we determined the average standing crop for each species in each of the four trophic state categories. If a species was missing in a lake, its standing crop was included as zero. A one-way analysis of variance was used to determine if there were significant differences in the standing crops among the four trophic categories.

To test the idea that the proportion of piscivorous species declines with increasing trophic state (Persson et al. 1988), we calculated the percent of the total fish biomass that consisted of piscivorous fish for the 60 lakes that had both littoral and open-water samples. Those fish considered piscivorous as adults were classified on the basis of the works by Pflieger (1975), Lee et al. (1981), and Becker (1983) and included Florida gar (*Lepisosteus platyrhincus*), longnose gar (*Lepisosteus osseus*), bowfin (*Amia calva*), chain pickerel (*Esox niger*), redfin pickerel (*Esox americanus americanus*), Atlantic needlefish (*Strongylura marina*), black crappie (*Pomoxis nigromaculatus*), and largemouth bass (*Micropterus salmoides*). Proportions should be useful in detecting relative changes in piscivorous activity; whereas absolute values would be overestimates because these species also feed on other food items.

Because of their importance to the sport fishery, we also calculated the weight of all centrarchids and largemouth bass in each lake's sample (as kilograms per hectare and as percentages of the total fish weight). For five important sport fish species we used the length–frequency data from the blocknet samples from the 60 lakes with weighted means of open-water and littoral fish to calculate relative stock densities (RSD-Q) for quality or larger fish following Gabelhouse (1984). For the warmouth (*Lepomis gulosus*) in each lake we divided the number of fish in the sample 15 cm or greater in length by the number greater than 8 cm long. The resulting RSD-Q value represents the proportion of the stock fish (those fish greater than 8 cm) that are of quality size (15 cm) or greater. The same lengths were used for the bluegill (*Lepomis macrochirus*) calculations. For the redear sunfish (*Lepomis microlophus*) we divided the number of fish larger than 18 cm by those greater than 12 cm; for the black crappie, the number greater than 20 cm by those greater than 12 cm;

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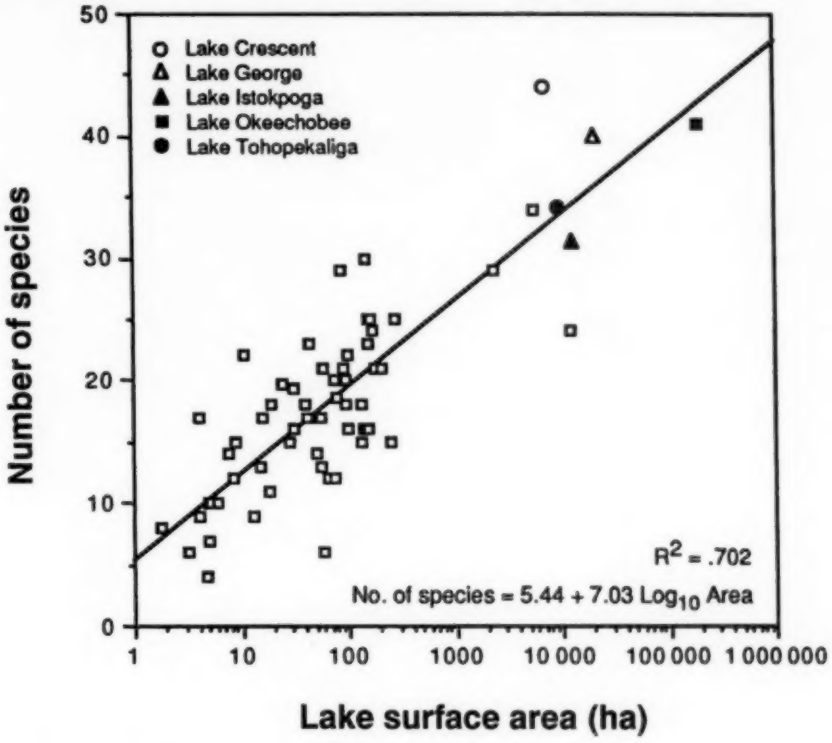
Fig. 3. Relationship between the number of species of fish per lake and the logarithm of lake surface area.



and for the largemouth bass, the number of fish greater than 32 cm by those greater than 20 cm. These are the same or close to the minimum sizes suggested by Gabelhouse (1984).

Water quality

For the 60 Florida lakes sampled by Canfield and Hoyer (1992), six water-quality sampling stations were established at each lake. Summer water samples were collected from six stations (three each in the littoral and pelagic zones) on a single date at the time the fish populations were sampled. Additional samples were collected from the three pelagic stations on two subsequent dates. Secchi disk measurements were made only at the open-water stations. Water was collected from just below the surface (0.5 m) in acid-cleaned Nalgene bottles. Samples were placed on ice and returned to the laboratory for analysis. The measurements that are used in this analysis included total phosphorus, determined with the methods of Murphy and Riley (1962) after persulfate oxidation (Menzel and Corwin 1965); total nitrogen, determined by a modified Kjeldahl technique (Nelson and Sommers 1975); and total chlorophyll *a* concentrations (uncorrected for phaeophytins), determined by filtering a measured portion of lake water through a Gelman type A-E glass fiber filter and then following the method of Yentsch and Menzel (1963) and the equations of Parsons and Strickland (1963).

Surface water samples (0.5 m) were collected from eight open-water stations in Lake Okeechobee. Secchi disk readings were made at the same time and sample analyses included total phosphorus, total nitrogen, and chlorophyll *a*. The station locations and analytical methods are discussed in James et al. (1995). The stations were sampled at least monthly and sometimes twice a month. For this study we calculated monthly averages for each variable for each station and then averaged the station means to find a lake average for each month. The lake average was taken as the mean of the monthly averages for the years 1989 through 1991. Water-quality data from the open waters of the other four large lakes were based on records and published reports of the Florida Game and Fresh Water Fish Commission (Moyer et al. 1992, 1993; Lange et al. 1993).

Statistical analysis

For tests of the effects of trophic state on fish standing crops in regression analyses, logarithms of total phosphorus, total nitrogen, algal chlorophyll *a*, and Secchi disk depth were used because these variables span several orders of magnitude and their expected errors increase with magnitude. Both linear and curvilinear fits were tried. The parametric statistical analyses (paired *t*-tests, bivariate correlation coefficients, and analysis of variance) were run according to Snedecor and Cochran (1980) using the JMP statistical package (SAS Institute 1994). Multiple regressions were run with the stepwise, forward selection mode (SAS Institute Inc. 1985). Chi-square analyses followed the procedures given in Siegel (1956). The 5% level of confidence was used to reject null hypotheses in all cases.

Results

Two-way analysis of variance showed no differences ($p < 0.05$) in concentrations of total phosphorus, total nitrogen, and chlorophyll *a* from open-water samples, littoral area samples, and averages of the two for the 60 original lakes. No differences ($p < 0.05$) were found in a comparison of our open-water samples from Lake Okeechobee and littoral samples (Phlips et al. 1993) from that lake. Thus, either littoral samples

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Table 3. Fish species collected in 65 Florida lakes ranked by frequency of occurrence.

Common name	Scientific name	No. of lakes	TP ($\mu g \cdot L^{-1}$)	CHL *a* ($\mu g \cdot L^{-1}$)	TN ($\mu g \cdot L^{-1}$)	Surface area (ha)	Standing crop: Whole lake ($kg \cdot ha^{-1}$)	Standing crop: Littoral only ($kg \cdot ha^{-1}$)	Lake size effect	Trophic state effect
Bluegill	*Lepomis macrochirus*	64	57	29	938	413	38.102		None	None
Largemouth bass	*Micropterus salmoides*	64	57	29	938	413	15.102		None	None
Warmouth	*Lepomis gulosus*	63	57	28	934	418	8.639		None	None
Mosquitofish	*Gambusia holbrooki*	51	68	33	1023	504	0.274		None	None
Redear sunfish	*Lepomis microlophus*	50	73	37	1132	537	15.067		More	More
Golden shiner	*Notemigonus crysoleucas*	47	76	38	1177	571	5.321		More	More
Brook silverside	*Labidesthes sicculus*	47	53	27	939	542	0.138		None	None
Lake chubsucker	*Erimyzon sucetta*	42	27	12	735	284	11.751		None	Less
Black crappie	*Pomoxis nigromaculatus*	42	84	42	1192	634	4.840		More	More
Swamp darter	*Etheostoma fusiforme*	40	31	14	724	283	0.033		None	None
Brown bullhead	*Ictalurus nebulosus*	34	95	43	1229	784	2.802		More	More
Yellow bullhead	*Ictalurus natalis*	33	32	29	961	737	1.290		None	None
Seminole killifish	*Fundulus seminolis*	32	79	42	1142	822	0.628		None	More
Golden topminnow	*Fundulus chrysotus*	32	22	11	688	156	0.344		None	Less
Bluespotted sunfish	*Enneacanthus gloriosus*	29	22	17	846	407	1.779		More	None
Bluefin killifish	*Lucania goodei*	29	26	20	814	414	0.729		More	None
Threadfin shad	*Dorosoma petenense*	26	82	40	1195	1 003	20.940		More	More
Gizzard shad	*Dorosoma cepedianum*	25	123	67	1599	1 104	66.074		More	More
Florida gar	*Lepisosteus platyrhincus*	25	47	23	883	480	2.682		More	None
Spotted sunfish	*Lepomis punctatus*	24	107	40	1169	522	2.626		More	More
Dollar sunfish	*Lepomis marginatus*	24	23	15	795	366	1.212		None	None
Bowfin	*Amia calva*	20	57	26	1015	240	4.830		More	None
Least killifish	*Heterandria formosa*	19	40	12	712	173	0.084		None	None
Lined topminnow	*Fundulus lineolatus*	19	10	6	422	43	0.190		Less	Less
Blue tilapia	*Tilapia aurea*	18	152	80	1818	1 380	12.731		More	More
Tadpole madtom	*Noturus gyrinus*	16	43	28	1128	1 468	0.279		More	None
White catfish	*Ictalurus catus*	15	122	40	1353	1 760	1.085		More	More
Taillight shiner	*Notropis maculatus*	15	67	61	1598	1 915	0.673		More	More
Sailfin molly	*Poecilia latipinna*	15	44	42	1316	1 919	0.085		More	More
Chain pickerel	*Esox niger*	13	17	11	728	332	4.232		None	None
Grass carp	*Ctenopharyngodon idella*	12	44	39	1170	1 096	17.211		None	None
Redfin pickerel	*Esox americanus americanus*	12	16	7	649	572	1.035		None	Less
Everglades pygmy sunfish	*Elassoma evergladei*	11	54	19	619	45	0.033		None	Less
Atlantic needlefish	*Strongylura marina*	8	87	112	2856	6 049	0.906		More	More
Flagfish	*Jordanella floridae*	8	20	7	800	136	0.347		More	None
Redbreast sunfish	*Lepomis auritus*	7	53	66	1570	1 176	1.419		None	None
Bream	*Lepomis* sp.	6	180	96	1976	156	1.267		None	None
Shiners	*Notropis* sp.	6	75	27	1094	993	0.120		None	None
Longnose gar	*Lepisosteus osseus*	5	81	102	2942	4 242	0.016		More	None
Pygmy killifish	*Leptolucania ommata*	5	9	4	503	31	0.002		None	None
Pirate perch	*Aphredoderus sayanus*	4	42	19	975	643	0.106		None	None
Striped mullet	*Mugil cephalus*	3	161	48	2477	65 963		52.537	None	None
Channel catfish	*Ictalurus punctatus*	3	144	24	2227	63 499		5.447	None	None
American eel	*Anguilla rostrata*	3	27	24	777	19	3.211		None	None
Sunshine bass	*Morone chrysops* × *Morone saxatilis*	3	87	112	2856	6 049	0.466		None	None
Flier	*Centrarchus macropterus*	3	22	16	853	792	0.185		None	None
Ladyfish	*Elops saurus*	2	195	56	2975	12 445		0.450	None	None
Hogchoker	*Trinectes maculatus*	2	195	56	2975	12 445		0.350	None	None
Clown goby	*Microgobius gulosus*	2	111	32	2990	89 645		0.211	None	None
Bay anchovy	*Anchoa mitchilli*	2	195	56	2975	12 445		<0.100	None	None
Sheepshead minnow	*Cyprinodon variegatus*	1	92	30	1480	173 000		0.310	None	None
Snook	*Centropomus undecimalis*	1	92	30	1480	173 000		0.179	None	None

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Table 3 (*concluded*).

Common name	Scientific name	No. of lakes	TP (μg·L^{-1})	CHL *a* (μg·L^{-1})	TN (μg·L^{-1})	Surface area (ha)	Standing crop: Whole lake (kg·ha^{-1})	Standing crop: Littoral only (kg·ha^{-1})	Lake size effect	Trophic state effect
Blackbanded sunfish	*Enneacanthus chaetodon*	1	11	4	462	85	0.104		None	None
Grey snapper	*Lutjanus griseus*	1	130	34	4500	6 290		0.100	None	None
Naked goby	*Gobiosoma bosc*	1	130	34	4500	6 290		0.100	None	None
Inland silversides	*Menidia beryllina*	1	92	30	1480	173 000		0.092	None	None
Pugnose minnow	*Opsopoeodus emiliae*	1	92	30	1480	173 000		0.006	None	None
Opossum pipefish	*Microphis brachyurus*	1	92	30	1480	173 000		0.002	None	None
Walking catfish	*Clarias batrachus*	1	92	30	1480	173 000		0.002	None	None
Atlantic croaker	*Micropogonias undulatus*	1	130	34	4500	6 290		<0.100	None	None
Silver jenny	*Eucinostomus gula*	1	130	34	4500	6 290		<0.100	None	None
American shad	*Alaso sapidissima*	1	260	79	1450	18 600		<0.100	None	None
Atlantic stingray	*Dasyatis sabina*	1	260	79	1450	18 600		<0.100	None	None
Gulf pipefish	*Syngnathus scovelli*	1	260	79	1450	18 600		<0.100	None	None
Pugnose shiner	*Notropis anogenus*	71	260	79	1450	18 600		<0.100	None	None

Note: For the lakes in which they were found, average values are given for the trophic state indicators total phosphorus (TP), chlorophyll *a* (Chl *a*), total nitrogen (TN), lake surface areas and whole lake standing crops (based on both littoral and open-water blocknets). For six of the lakes only littoral standing crop data are available. Chi-square or Fisher exact probability tests were used to determine if fish species were more or less abundant in larger rather than smaller lakes or in eutrophic–hypertrophic lakes rather than oligotrophic–mesotrophic lakes. More, significant increase; less, significant decrease; none, no difference.

or a combination of littoral and open-water samples can be used to characterize lakes in a broad survey of this type.

Total phosphorus, total nitrogen, and chlorophyll *a* concentrations ranged over two or three orders of magnitude (Table 1). For example, chlorophyll *a* ranged from 1 to 241 mg·m^{-3}. There were no significant correlations between the trophic state indicators and lake mean depth. This probably results from the very small range of mean depths in these Florida lakes (0.6–5.8 m; Table 1). There was a tendency for the larger lakes to be more eutrophic. We found weak positive correlations ($p < 0.05$) between total phosphorus, total nitrogen, and chlorophyll *a* with the logarithms of surface area ($r^2 = 0.09$, 0.26, and 0.09, respectively) and a negative correlation with Secchi disk depth ($r^2 = 0.22$).

Chlorophyll *a* concentrations in these lakes were highly correlated ($p < 0.01$) with both total phosphorus and total nitrogen (Table 2). Because total phosphorus and total nitrogen were both correlated ($p < 0.01$) with each other (Table 2), these data do not indicate which is the limiting element. The Secchi disk transparency is highly correlated with chlorophyll *a*, total phosphorus, and total nitrogen ($p < 0.01$; Table 2). For these lakes as a group the chlorophyll *a* concentration largely determines the water transparency rather than suspended solids and humic materials, although there are exceptions in some individual lakes.

The standing crop of fish based on a weighted average of catches in both littoral and open-water blocknets averaged 131 kg·ha^{-1} (range 7–683 kg·ha^{-1}). For the 60 lakes with both littoral and open-water data the standing crops of fish increased with increases in chlorophyll *a*, total phosphorus, and total nitrogen and decreased with increases in Secchi disk transparency (Fig. 2). The correlations were significant ($p < 0.01$) though the r^2 values (0.27, 0.24, 0.28, and 0.27, respectively) indicated that trophic state explained only about one fourth of the variance in fish crops. There was no significant correlation between the standing crop of fish and lake surface area. Other factors and experimental error in sampling may be responsible for a great portion of the observed variance in the standing crop estimates. Five lakes only had estimates of littoral fish standing crops, so they were not included in the correlation analysis. The points for littoral fish crops in those five lakes are plotted in the same graphs, however, to illustrate that they fall within the same scatter as the points for the other 60 lakes (Fig. 2).

On average there were 18 species of fish in each lake (range 4–44 species; Table 1). The larger the lake the more species of fish that were found. Lake surface area (Fig. 3) explained 70% of the variance in species numbers. A number of marine species (e.g., Atlantic stingray (*Dasyatis sabina*), bay anchovy (*Anchoa mitchilli*), Atlantic croaker (*Micropogonias undulatus*), etc.) occurred in some of the larger lakes with oceanic connections. A reanalysis with those species removed still yielded an r^2 of 0.67. Species richness was weakly correlated with total phosphorus, total nitrogen, chlorophyll *a*, and Secchi depth ($r^2 = 0.05$, 0.20, 0.03, and 0.25, respectively) with a positive relationship for the first three variables and a negative relationship for the Secchi disk transparency. However, in a stepwise, multiple regression in the forward mode using logarithms of surface area, total phosphorus, total nitrogen, chlorophyll *a*, and Secchi disk transparency as independent variables, log surface area was the only significant variable selected. There was no indication that the number of fish species declined with increasing trophic state in these lakes.

Sixty-five fish species were collected in the study lakes (Table 3). Bluegill, largemouth bass, warmouth, mosquitofish (*Gambusia holbrooki*), and redear sunfish were found in 75–98% of the lakes, whereas 15 other species were collected in only a single lake. Significant differences in their distributions between lakes of different trophic states were found for 18 species (Fig. 4). Thirteen of the fish species were more likely to be present in lakes of higher trophic states while only

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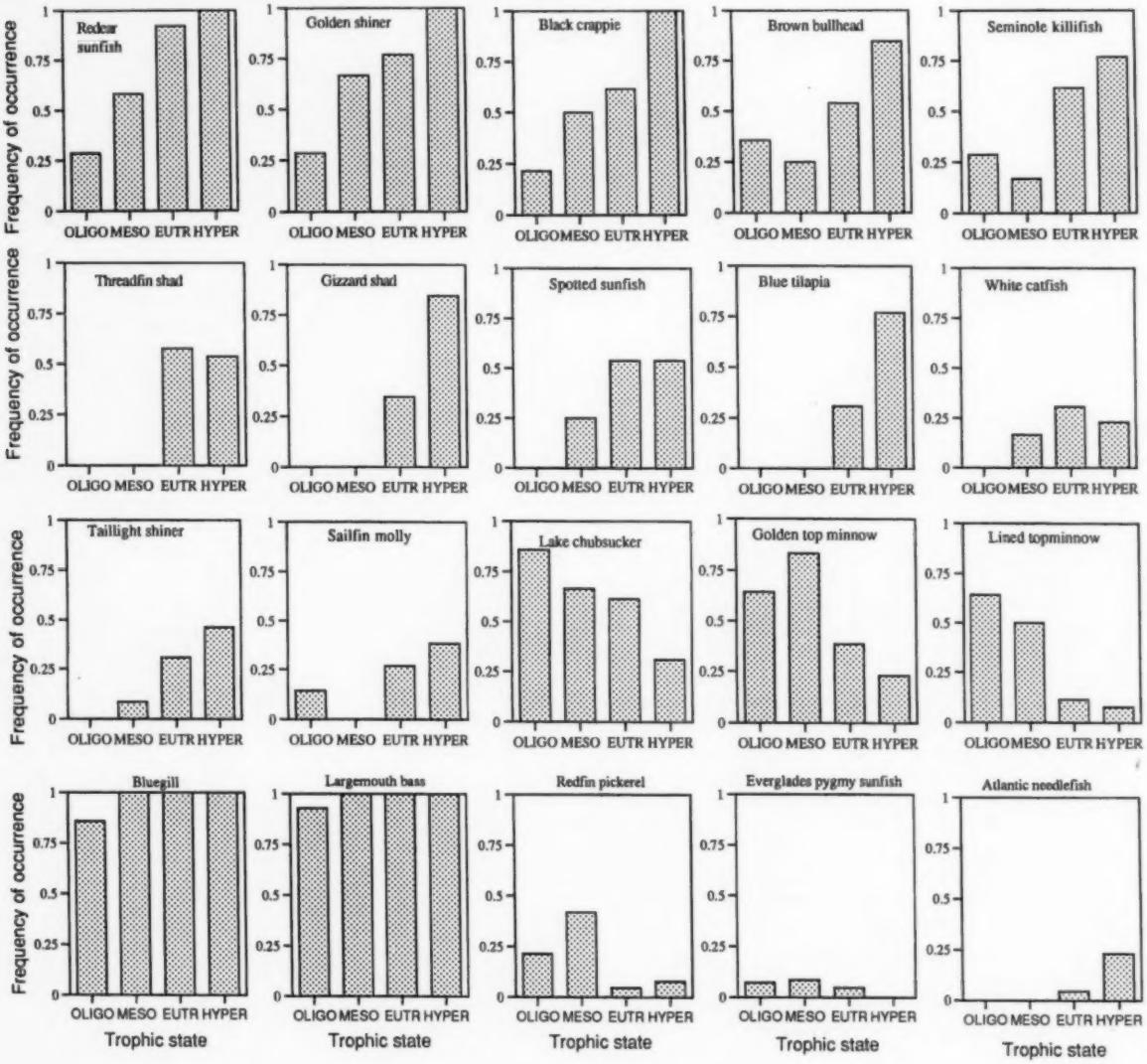
Fig. 4. Fraction of lakes of different trophic state in which various species of fish were present. For each of these species, except the bluegill and largemouth bass, there was a significant difference between the fraction found in oligotrophic–mesotrophic lakes and the fraction found in eutrophic–hypereutrophic lakes.



five species, the lake chubsucker (*Erimyzon sucetta*), golden topminnow (*Fundulus chrysotus*), lined topminnow (*Fundulus lineolatus*), redfin pickerel, and Everglades pygmy sunfish (*Elassoma evergladei*), were less likely to be found in eutrophic–hypereutrophic lakes than oligotrophic–mesotrophic lakes (Table 3). Another indication of the trophic state preferences for these species can be derived by looking at the average values for the trophic state indicators for the lakes in which they were found (Table 3) relative to the averages for the entire sample (Table 1). For example, the redfin pickerel was most commonly found in lakes with low nutrient concentrations while the blue tilapia (*Tilapia aurea*) was more likely to be in lakes with higher nutrient concentrations.

A similar test was made of the effect of lake size on the distribution of individual fish species. The lakes were divided into small lakes (<100 ha) and large lakes (>100 ha), and a chi-square or Fisher exact probability test (Siegel 1956) was used to test the distribution of presence or absence. Nineteen species were more likely to be found in large lakes while only one was more likely to be found in small lakes (Table 3). In most cases the fish most likely to be found in eutrophic–hypereutrophic lakes were the same species that were more likely to be found in large lakes. Because our larger lakes tended to be more eutrophic, it is not possible to sort out completely the effects of lake size and trophic state.

Standing crops of individual species increased with trophic

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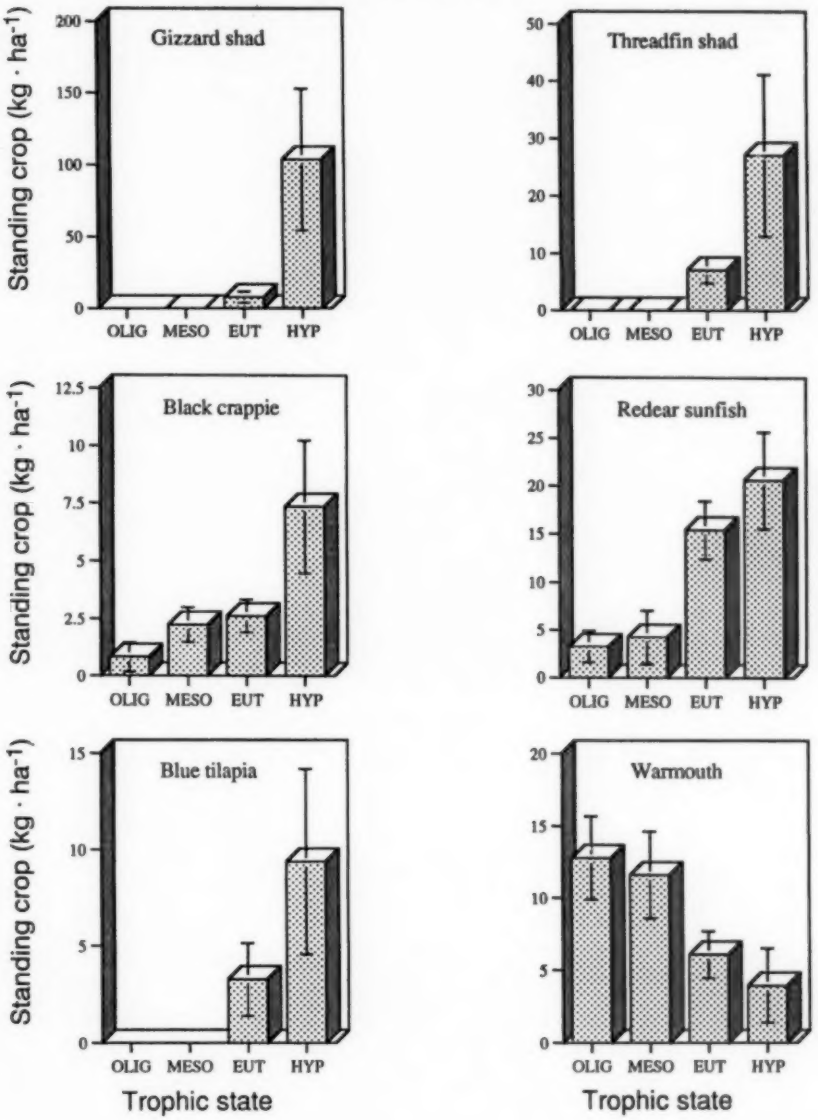
Fig. 5. Average standing crops with standard errors for fish in groups of lakes of different trophic state. For each of these species a one-way analysis of variance showed significant differences ($p < 0.05$) among standing crops for the four trophic categories.



state ($p < 0.05$) for the gizzard shad (*Dorosoma cepedianum*), threadfin shad (*Dorosoma petenese*), black crappie, redear sunfish, and blue tilapia while the warmouth tended to have higher standing crops in lakes of lower productivity (Fig. 5). The increases in biomass for the gizzard shad, the threadfin shad, and the blue tilapia are particularly noteworthy. All were practically absent from oligotrophic–mesotrophic lakes (Fig. 4) but increased in both frequency of occurrence and average standing crop in eutrophic and especially hypereutrophic lakes (Fig. 5). The average standing crop for the gizzard shad for lakes in which it was found (66 kg·ha^{-1}) was the highest for all species encountered while the standing crop for the threadfin shad (21 kg·ha^{-1}) ranked third behind the bluegill (38 kg·ha^{-1}). The blue tilapia showed a similar pattern in both frequency of occurrence (Fig. 4) and average standing crop (Fig. 5) as the two shad species.

The piscivorous species as a group averaged about 22% of the total fish biomass in our lakes and ranged from 0 to 73%. The absolute weights of the piscivore group tended not to change with increasing trophic state (Table 4). However, when expressed as a percentage of the total biomass the relative importance of this group declined as the lakes became more productive, though the r^2 values are quite small (0.03–0.12). On average the percentages of piscivorous species for

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Table 4. Pearson's correlations (r^2) between several indices of lake trophic state and several measures of fish population composition and quality for the 60 Florida lakes with data on both open-water and littoral fish.

	Log total phosphorus	Log total nitrogen	Log chlorophyll *a*	Log Secchi depth
Total biomass minus shad	0.14**	0.14**	0.14**	(–) 0.07*
Weight of piscivores	0.01	0.06*	0.02	(–) 0.01
Weight of centrarchids	0.14**	0.14**	0.13**	(–) 0.06*
Weight of largemouth bass	(–) 0.01	(–) 0.00	(–) 0.00	0.00
Percentages based on total biomass				
Piscivores	(–) 0.09*	(–) 0.03	(–) 0.12**	0.07*
Centrarchids	(–) 0.10*	(–) 0.06*	(–) 0.19**	0.16**
Largemouth bass	(–) 0.21**	(–) 0.14**	(–) 0.17**	0.17**
Percentages based on total biomass minus biomass of shad				
Piscivores	(–) 0.02	(–) 0.00	(–) 0.02	0.01
Centrarchids	0.01	0.01	0.00	0.00
Largemouth bass	(–) 0.14**	(–) 0.10*	(–) 0.09*	0.11**
RSD-Q				
Black crappie	(–) 0.27**	(–) 0.35**	(–) 0.23**	0.23**
Bluegill	(–) 0.01	(–) 0.03	(–) 0.01	0.02
Largemouth bass	0.12**	0.16**	0.22**	(–) 0.10*
Redear sunfish	0.01	(–) 0.00	0.00	(–) 0.02
Warmouth bass	0.08*	0.04	0.14**	(–) 0.13**

Note: The percentages were calculated on both the total weight of fish and the total weight minus the weights of gizzard shad and threadfin shad. The minus signs in parentheses indicate negative relationships. The RSD-Q represents the proportion of the fish stock greater than a minimum size (see text). Curvilinear regressions were also tried, but they did not result in improved fits for these data.

*Significant at the 5% level.

**Significant at the 1% level.

oligotrophic, mesotrophic, eutrophic, and hypereutrophic lakes were 25, 28, 21, and 11%, respectively, of the total biomass. One of the most important piscivores, the largemouth bass, on average, made up 15% of the fish biomass with a range of from 0 to 69%. The absolute biomass of the largemouth bass showed no changes with trophic state (Table 4), though their percentage of the total biomass became less abundant at higher trophic states. On average the percentages of largemouth bass by weight in oligotrophic, mesotrophic, eutrophic, and hypereutrophic lakes were 20, 17, 16, and 4%, respectively, of the total biomass. The centrarchids, on average, made up 66% of the total biomass with a range from 15 to 99%. They likewise showed a pattern of increasing absolute biomass with increases in trophic state (Table 4) but decreasing in percent biomass in lakes of higher trophic states.

Because of the large increase in standing crops of gizzard shad and threadfin shad found in eutrophic and hypereutrophic lakes, we analyzed the data to see if these increases might be responsible for the drops in the relative proportions of the piscivores, centrarchids, and largemouth bass. The biomasses of these two shad species were subtracted from the total fish biomass to derive a new biomass total that did not include the shad species. This new total also showed increases with increases in trophic state (Table 4); however, both the percent piscivores and percent centrarchids now showed no correlation with trophic state while the percent largemouth bass continued to decrease in lakes at higher trophic levels. This suggests that the addition of large standing crops of gizzard and threadfin shad to the total fish biomass was responsible for the decrease in the proportions of the piscivores and centrarchids as groups.

The largemouth bass is one of the most important fish species in terms of occurrence because it was found in 64 of 65 of our study lakes and ranked fifth in average standing crop for the lakes in which it was found (Table 2). It showed no pattern of change in standing crop with changes in lake trophic state for this group of lakes but did show a decline in percentage of total biomass as lakes became more eutrophic. At the same time, however, the proportion by number of largemouth bass of quality size or greater as defined by Gablehouse (1984) increased with increasing trophic state (Table 4). Two other fish, the bluegill and warmouth, were also very common, being found in 64 and 63 of our lakes, respectively. The bluegill showed no changes in average standing crops or in proportions of larger fish with increases in trophic state (Table 4) while the warmouth showed decreases in standing crop (Fig. 5) but increases in the proportion of larger sized individuals with increases in trophic state (Table 4). The redear sunfish showed increases in frequency of occurrence (Fig. 4) and increases in average standing crop (Fig. 5) but no change in RSD-Q (Table 4) as lakes increased in productivity. The other important centrarchid, the black crappie, also became more common (Fig. 4) and had larger standing crops (Fig. 5) in more eutrophic lakes but had a smaller proportion of quality-sized fish (Table 4) in the richer lakes.

Discussion

The fish populations in the Florida lakes in this study fit some of the patterns found for other lakes discussed in the fisheries literature. There is an increase in the total standing crops of fish as the concentrations of total phosphorus, total nitrogen, and chlorophyll *a* increase and as the Secchi depth decreases. Similar results were found by Bays and Crisman (1983) for 30

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Florida lakes. Canfield and Hoyer (1992) also found correlations between fish standing crops and trophic state for 60 Florida lakes when they took into account the standing crops of aquatic macrophytes in addition to phytoplankton in determining trophic state. On average the standing crops increased about one order of magnitude from the oligotrophic to the hypereutrophic Florida lakes in this study with no sign of a decrease in the most hypereutrophic lakes. There is considerable unexplained variance (about 75%) in these relationships due in part to other factors influencing fish crops and the practical sampling problems of estimating the biomass of wild fish populations. For this reason, predictions based on these relationships are imprecise; however, holding all other things constant, the standing crop of fish in Florida lakes should increase as they become more eutrophic.

Increases in trophic state did not result in a decrease in the number of fish species per lake. The lake surface area accounted for most of the variance in total species numbers, and once area was taken into account, there was no correlation between the number of fish species per lake and the various trophic state indices. The finding that larger lakes have a greater species richness than smaller lakes has been documented in comparisons of lakes throughout the world, including North America and Africa (e.g., Barbour and Brown 1974) and in Florida (Keller and Crisman 1990). A number of studies have documented this species–area relationship and tested hypotheses such as equilibria related to colonization and extinction, as suggested by theories of island biogeography and habitat diversity (Browne 1981; Barbour and Brown 1974; Eadie et al. 1986; Magnuson 1976; Tonn and Magnuson 1982). Studies investigating species diversity and fish community assemblages have also looked at factors such as size, pH, plant nutrients, latitude, and insularity and applied statistical techniques to determine the factors that influence species richness (Connor and McCoy 1979; Tonn and Magnuson 1982; Keller and Crisman 1990). Results of regression analyses for lakes in Florida and on data from other parts of North America by Keller and Crisman (1990) show that lake area explains the largest proportion of the variance in fish species richness.

Only five species showed decreases in frequency of occurrence with increasing trophic state, while all other species either stayed the same or increased in frequency of occurrence as lakes became more eutrophic. A similar pattern was found for average standing crops of individual species in lakes of different trophic states. Most species showed no significant change in biomass with changes in trophic state, while one species, the warmouth, decreased. Five species, the gizzard shad, threadfin shad, black crappie, redear sunfish, and blue tilapia, showed increases in standing crops with increasing trophic state. Bays and Crisman (1983) also noted the increased standing crops of shad in Florida lakes. For the recreationally important centrarchids the only negative changes noted with higher trophic states were lower standing crops of warmouth and lower proportions of larger sized black crappie. On the positive side the centrarchids as a group increased in biomass, the redear sunfish and black crappie increased in average standing crop, and the largemouth bass had a higher proportion of larger fish in the more eutrophic lakes.

Persson et al. (1988) found that the piscivorous fish in 15 Swedish lakes they had studied decreased in relative abundance as their lakes became more eutrophic. The piscivores made up about 70% of the biomass in their oligotrophic lakes and dropped to about 7% in lakes of mesotrophic or higher trophic state. They thought that in very productive lakes with decreased habitat heterogeneity their primary piscivore, the perch (*Perca fluviatilis*), faced increased competition from cyprinids and that this had a negative effect on the recruitment of perch. We also found declines in the relative proportions of piscivores to other fish groups when we looked at lakes of increasing trophic state; however, the magnitude of the decrease was not as great as that found for the Swedish lakes. In our case there was no decrease in the absolute standing crop of the piscivores, but rather higher standing crops of gizzard shad and threadfin shad in eutrophic and hypereutrophic lakes reduced the relative importance of the piscivores.

We could only study the relationships between fish and trophic state over a cross section of lakes of varying trophic state rather than in individual lakes undergoing eutrophication over time. This places a limit on predictions that can be made for Lake Okeechobee or other lakes that may show changes in trophic state due to changes in nutrient inputs resulting from land use changes in their watersheds. Because of the scatter in our data, due in part to the many unexplained factors influencing fish populations and the problems in obtaining precise standing crop information for fish, we would have a problem in making a precise prediction of fish standing crops for an unknown Florida lake given information on basic water chemistry. On the other hand there were some general patterns in the relationships between lake trophic state and the total biomass, species richness, species composition, and species standing crops of fish in Florida lakes that should be useful in a predictive manner. First, the number of fish species in a lake seemed to be determined primarily by the size of the lake and not the trophic state, so we should not expect dramatic changes in total species numbers as lakes become more or less eutrophic. Second, there might be shifts in species composition with changes in trophic state, though only a few species showed significant changes in their standing crops. In particular the recreationally important centrarchids did not show dramatic changes over the trophic spectrum. Third, we did not identify any critical points on the trophic spectrum that caused dramatic changes in fish abundance and standing crops; there was nothing comparable with the loss of dissolved oxygen in the hypolimnion of a eutrophying northern lake. Last, the trophic indicators for our sample of lakes ranged over 2 or 3 orders of magnitude. The expected change in trophic state of a Florida lake that is already in the eutrophic to hypereutrophic category will likely be much less, so changes in the fish population due to changes in trophic status are likely to be small, and any changes in fish populations brought upon by changes in trophic state will be overshadowed by the many other variables that influence fish populations from year to year.

Since these findings do not fit the expectations of several earlier studies in the literature, it is worth looking at how these Florida lakes might differ from the lakes in those earlier studies. One major difference is that many of the lakes where eutrophication has been cited as the cause for changes in fish populations have been deep, stratified lakes in temperate regions where salmonid populations have been reduced or eliminated (Larkin and Northcote 1969). The loss of dissolved oxygen in the hypolimnion of stratified lakes is an important factor in reducing the available habitat for these species of fish

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and their preferred foods (Colby et al. 1972). Florida lakes, however, are shallow, do not have cold hypolimnia, and lack salmonid species. Winterkills under the ice in the winter are also related to the impacts of eutrophication on fish community structure (Lee et al. 1991), but again this situation would not be relevant in Florida's climate. Lastly, in the case of the 22 lakes studied by Kautz (1980), his plot of sport fish biomass versus trophic state as determined by total nitrogen content did indicate a downward trend for hypereutrophic lakes. However, when he grouped the lakes by trophic state he found that the standing crops of sport fishes for oligotrophic, mesotrophic–eutrophic, and hypereutrophic lakes were 52, 89, and 65 $kg \cdot ha^{-1}$, respectively, but these values were not statistically different from each other. Thus, those data did not support the contention that sport fish biomass declined with increasing trophic state in Florida lakes. According to our study, Florida lakes with up to 240 $mg \cdot m^{-3}$ of algal chlorophyll *a* show no significant decrease in biomass of important sport fish except the warmouth.

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The effect of incremental pH recovery on the Lake 223 phytoplankton community

D.L. Findlay and S.E.M. Kasian

Abstract: Experimental acidification of Lake 223 from pH 6.7 to 5.1 caused dramatic effects on the phytoplankton community, similar to those recorded for systems anthropogenically affected. Species diversity decreased, biomass increased, and dominance shifted from chrysophytes to dinoflagellates. Increasing the pH from 5.0 to 5.8 stimulated very little recovery response. Biomass and composition remained unaltered, but species diversity increased as pH increased. Elevating pH above 6 stimulated a strong recovery of the Lake 223 phytoplankton community. Species diversity continued to increase as pH increased. Although chrysophyte biomass increased, total biomass began to decrease to pre-acidification levels because of declined abundances of cyanobacteria, dinoflagellates, chlorophytes, and cryptophytes. The size structure of the phytoplankton began to resemble more closely that seen in communities of reference lakes.

Résumé : L'acidification expérimentale de pH 6,7 à pH 5,1 du lac 223 a causé chez la communauté phytoplanctonique des effets saisissants semblables à ceux observés dans les systèmes subissant des effets d'origine anthropique. Il y avait diminution de la diversité des espèces, accroissement de la biomasse et déplacement de la dominance des chrysophytes vers les dinoflagellés. L'accroissement du pH de 5,0 à 5,8 n'entraînait qu'une très faible réponse de récupération. La biomasse et la composition restaient inchangées, mais la diversité des espèces augmentaient au fur et à mesure qu'augmentait le pH. L'augmentation du pH au-dessus de 6 entraînait une forte récupération de la communauté phytoplanctonique du lac 223. La diversité des espèces continuait à augmenter au fur et à mesure qu'augmentait le pH. La biomasse des chrysophytes augmentait, mais la biomasse totale commençait à diminuer jusqu'aux niveaux pré-acidification en raison de la diminution d'abondance des cyanobactéries, des dinoflagellés, des chlorophytes et des cryptophytes. La structure de la taille de la communauté phytoplanctonique commençait à ressembler à celle des communautés des lacs de référence.
[Traduit par la Rédaction]

Introduction

The damage to aquatic and terrestrial habitat by acidification has been well documented in North America and Europe (Schindler et al. 1985, 1991; Bresser and Salomons 1989). Much of the aquatic acidification research in Canada was influential in the drafting of the joint emissions reduction program between Canada and the United States that reduced SO_2 emissions by 50% by 1994. With this legislation came the expectation that acid-impacted ecosystems would recover to their natural states. Hutchinson and Havas (1986), Kelso and Jefferies (1988), and McNicol and Mallory (1994) documented rapid recovery from acidification in water chemistry, as a result of reduced SO_2 inputs around the Sudbury, Ont., area in the 1970s–1980s. Documentation on the recovery of biological parameters has been less consistent. Kelso et al. (1990) concluded that recovery of lakes in the Sudbury area could occur based on re-establishment of fish populations, while Nicholls et al. (1992) documented the recovery of seven Ontario lakes that demonstrated an increasing number of phytoplankton taxa as pH increased. Dixit et al. (1989) used a paleolimnological study of Swan Lake (near Sudbury) to document recovery of the plankton community. However, Martin et al. (1986) concluded that, although water quality of previously acidified lakes has improved in eastern North America, both forest and aquatic biological situations are far from acceptable. The problem with assessing aquatic ecosystem recovery is being able to define the critical parameters of biological recovery (Havas 1989). We here address recovery in the phytoplankton community.

Lake 223, at the Experimental Lakes Area, in northwestern Ontario, Canada, was artificially acidified from pH 6.7 to ~5.0 from 1976 to 1983 (Findlay and Kasian 1986). Beginning in 1984 the lake pH was allowed to increase incrementally in a controlled recovery (Findlay and Kasian 1990), and by 1991 the pH was >6. Chemical monitoring of Lake 223 indicated an immediate response to increased pH indicating a progression to recovery (Schindler et al. 1991). The fish populations in Lake 223 that survived acidification resumed reproduction and lake trout (*Salvelinus namaycush*) condition increased as pH increased from 1984 to 1989 (Schindler et al. 1991). Chang and Malley (1989) concluded that the zooplankton community had only partially recovered in Lake 223 after the pH had been increased to 5.5 from 5.1 over four consecutive years. Findlay and Kasian (1990) showed that the Lake 223 phytoplankton community did not immediately recover to its natural state at a rate similar to the aquatic chemical parameters when pH was increased to 5.8.

This paper continues the analysis of the Lake 223 phytoplankton community by Findlay and Kasian (1986, 1990), documenting responses that have occurred from 1991 to 1994

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D.L. Findlay[1] and S.E.M. Kasian. Department of Fisheries and Oceans, Central and Arctic Region, Freshwater Institute, 501 University Crescent, Winnipeg, MB R3T 2N6, Canada.

[1] Author to whom all correspondence should be addressed.

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when the pH was increased above 6. Changes in species composition, species diversity, total biomass, and community size structure in relation to increasing pH are examined. Data from recovery years in experimental Lake 223 will be compared with pre-acidification data and with contemporaneous data from two reference lakes.

Methods

Study site

Lake 223 is a dimictic, oligotrophic lake situated within the Experimental Lakes Area (ELA) (49°41′N, 93°41′W). The lake has a surface area of 27.3 ha, a mean depth of 7.2 m, and a maximum depth of 14.4 m. Water renewal times during the period of record varied from 4.3 to 25.4 years. There is an outflow and a single inflowing stream from ultra-oligotrophic Lake 224. The 260-ha watershed surrounding Lake 223 is covered by a boreal forest of jack pine (*Pinus banksiana*), red pine (*Pinus resinosa*), and black spruce (*Picea mariana*). Prior to acidification the chemical regime was typical for lakes in this area (Schindler et al. 1980). In 1974 and 1975, average suspended C was 0.6 $mg \cdot L^{-1}$, pH was 6.7, and conductivity was 21 $\mu S \cdot cm^{-1}$ (Prokopowich 1979). From 1976 to 1981 the epilimnetic pH of Lake 223 was reduced to 5.0 (Table 1) in a controlled stepwise manner and held at that level from 1981 to 1983 by additions of H_2SO_4 (Cruikshank 1984). From 1984 to 1994, Lake 223 was experimentally manipulated through several phases of recovery (Table 1). Phases were defined as (*i*) premanipulation period, 1974–1975; (*ii*) acidification, pH decreased from 6.5 to 5.0; (*iii*) early recovery to pH 5.4–5.5; (*iv*) recovery to pH 5.8; and (*v*) recovery to pH >6.

Lakes 224 and 239 are oligotrophic, unmanipulated, reference lakes that are chemically typical of the ELA (Armstrong and Schindler 1971; Prokopowich 1979) and have phytoplankton communities typical for oligotrophic lakes in this area (Findlay and Kasian 1990, 1991; Kasian et al.[2]). They are 25 and 56 ha in area and have maximum depths of 25 and 30 m, respectively (Cleugh and Hauser 1971), with water renewal times similar to Lake 223 (K. Beaty, Department of Fisheries and Oceans, Winnipeg, Man., personal communication).

Phytoplankton sampling and enumeration

Phytoplankton samples were obtained from a desired stratum (epilimnion or metalimnion) using an integrating sampler (Shearer 1978). The depth boundaries of the stratum to be sampled were determined from temperature and light (Shearer 1978). For the purpose of this paper, only the epilimnion will be discussed. The sampler, equipped with a 2-L bottle, was lowered and raised through the stratum allowing the bottle to fill. Samples were collected every 2 weeks during the ice-free season from the three lakes.

Over the 20-year period the methods of preservation and enumeration have been constant. A 125-mL aliquot was subsampled and preserved in acid Lugol's solution for archiving, identification, and enumeration of phytoplankton. A 10-mL aliquot of a Lugol's preserved sample was gravity settled for 24 h. Identification and counts were performed on an inverted microscope at magnifications of 125×, 400×, and 1000× (oil) with phase-contrast illumination. All counts were done by the Ütermöhl technique as modified by Nauwerck (1963).

Cell sizes were routinely measured and averages were calculated from up to 50 individuals of each species in each sample. Measurements were done on the chloroplast and not necessarily on a whole cell (e.g., the lorica was not included in the measurements of *Dinobyron* spp.). Colony volumes were calculated from the volume of one cell multiplied by the estimated number of cells in the colony (Rott 1981). Cell density was converted to wet weight biomass by applying the geometric formula best fitted to the cell shape (Vollenweider 1968; Rott 1981) and assuming a specific gravity of 1 for the cellular mass.

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Table 1. Target and ice-free mean epilimnetic (epi) pH values for Lake 223 1974–1994.

Year	Phase	Target pH	Mean epi pH
1974	1 Natural		6.7
1975			6.6
1976	2 Acidification		6.5
1977		6.00	6.1
1978		5.75	5.9
1979		5.50	5.6
1980		5.25	5.6
1981		5.00	5.0
1982		5.00	5.1
1983		5.00	5.1
1984	3 Recovery to pH 5.5	5.40	5.4
1985		5.40	5.5
1986		5.40	5.5
1987		5.40	5.4
1988	4 Recovery to pH 5.8	5.80	5.8
1989		5.80	5.8
1990		5.80	5.8
1991	5 Recovery to pH >6	6.10	6.1
1992		6.10	6.2
1993		6.10	6.2
1994		none	6.7

Note: Phase refers to changes in pH regime.

Chemical measurements

Dissolved and suspended fractions of carbon (C), nitrogen (N), and phosphorous (P) were measured; the totals for carbon (TC), nitrogen (TN), and phosphorus (TP) are the sums of the fractions. Chlorophyll was measured fluorometrically. Analytical methods used for the determination of all elements are described by Stainton et al. (1977).

Data manipulation

Simpson's diversity index was chosen to quantify changes in species numbers relative to cell abundance. Washington (1984) concluded that this was the best index available for aquatic populations even though it was biased towards dominant species.

Data presented for lakes 223, 224, and 239 are epilimnetic, ice-free season, time-weighted, annual means. The phytoplankton population from Lake 223 was compared with populations from reference lakes 224 and 239 to separate experimental responses from temporal trends. A repeated measures, nested design analysis of variance (ANOVA) was performed on annual means of specified parameters (Table 2) using SAS (SAS Institute Inc. 1985). The procedure tested two hypotheses: P_t is the probability that the year-to-year trend in Lake 223 is equal to the year-to-year trend in reference lakes; P_m is the probability that the overall mean in Lake 223 is equal to the overall mean in the reference lakes. To better detect when changes in the phytoplankton community occurred in relation to pH, separate ANOVAs were performed on each phase of the experiment (Table 1).

Community changes in Lake 223 were assessed by principal components analysis (SAS Institute Inc. 1985). Biomass of the seven major taxonomic groups (cyanobacteria, chlorophytes, euglenophytes, chrysophytes, diatoms, cryptophytes, and dinoflagellates) was computed for lakes 223, 224, and 239 and the correlation matrix was analyzed.

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Table 2. Results of analysis of variance of phytoplankton populations in Lake 223 versus those in reference lakes.

	Phase 2		Phase 3		Phase 4		Phase 5	
	P_t	P_m	P_t	P_m	P_t	P_m	P_t	P_m
Total biomass	0.055	0.175	0.007*	0.179	0.071	0.125	0.002*	0.028*
Cyanobacteria	0.008*	0.049*	0.180	0.208	0.007*	0.007*	0.601	0.414
Chlorophyte	0.001*	0.221	0.003*	0.299	0.021*	0.238	0.234	0.472
Chrysophyte	0.157	0.323	0.006*	0.385	0.747	0.767	0.006*	0.061
Diatom	0.587	0.625	0.865	0.417	0.471	0.350	0.017*	0.133
Cryptophyte	0.052	0.375	0.432	0.243	0.571	0.720	0.805	0.919
Dinoflagellate	0.001*	0.039*	0.005*	0.037*	0.002*	0.002*	0.0001*	0.033*
Diversity	0.016*	0.016*	0.276	0.018*	0.463	0.278	0.591	0.0001*
Size fraction of biomass								
0–10 μm	0.632	0.048*	0.086	0.291	0.480	0.697	0.033*	0.115
10–30 μm	0.612	0.027*	0.432	0.305	0.513	0.665	0.045*	0.005*
>30 μm	0.032*	0.196	0.003*	0.207	0.070	0.131	0.0009*	0.046*

Note: Phase 2 (1976–1983) is the acidification phase (pH 6.5–5.0), and phase 3 (1984–1987; pH 5.4–5.5), phase 4 (1988–1990; pH 5.8), and phase 5 (1991–1994; pH 6.1–6.6) are the incremental recovery phases. Values with an asterisk are a significant response at $P < 0.05$. P_t, probability that the trend over time in Lake 223 was equal to the average trend over time in the reference lakes; P_m, probability that the overall mean in Lake 223 was equal to the overall mean in the reference lakes.

Fig. 1. Cumulative percent composition of epilimnetic phytoplankton averaged monthly for each of phases 1–4 (1974–1975, 1976–1983, 1984–1987, and 1988–1990) and 1991–1994 for experimental Lake 223 and reference lakes 224 and 239.

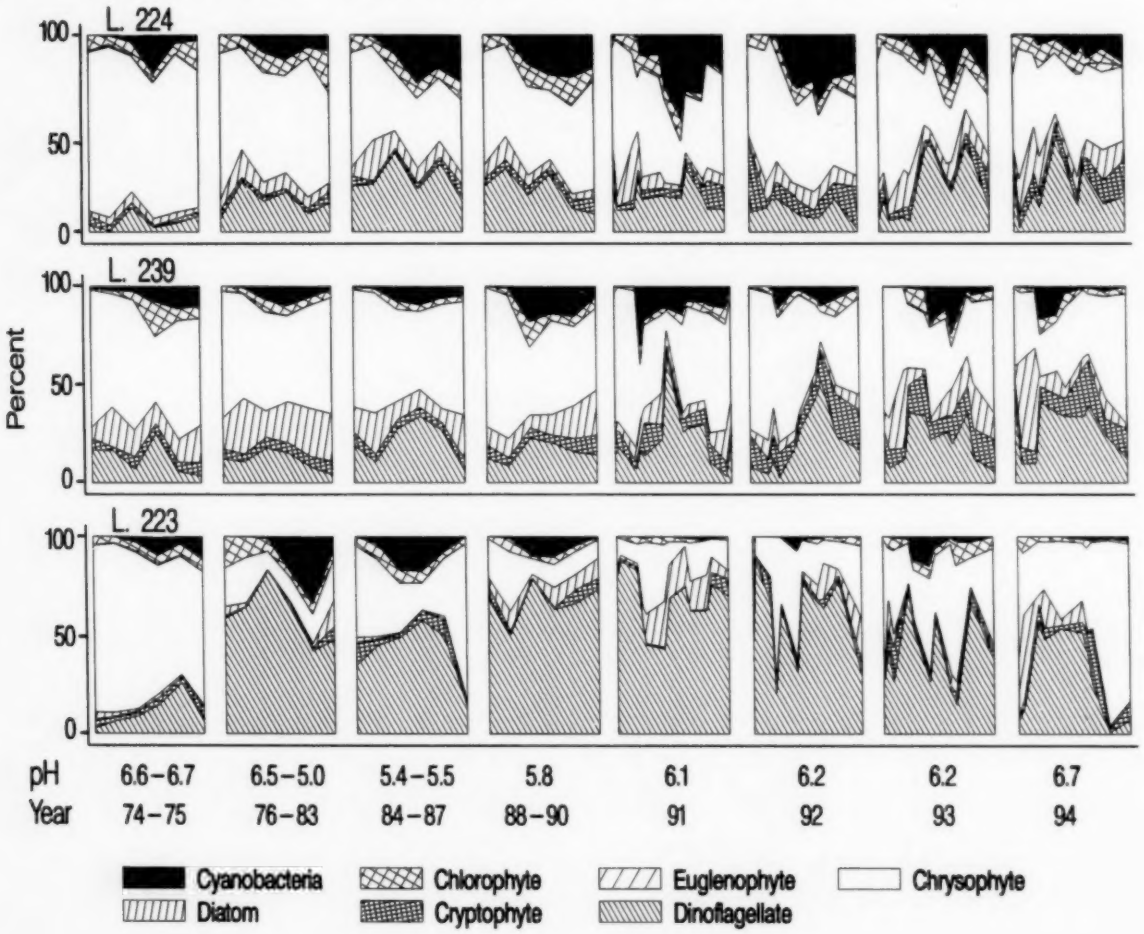

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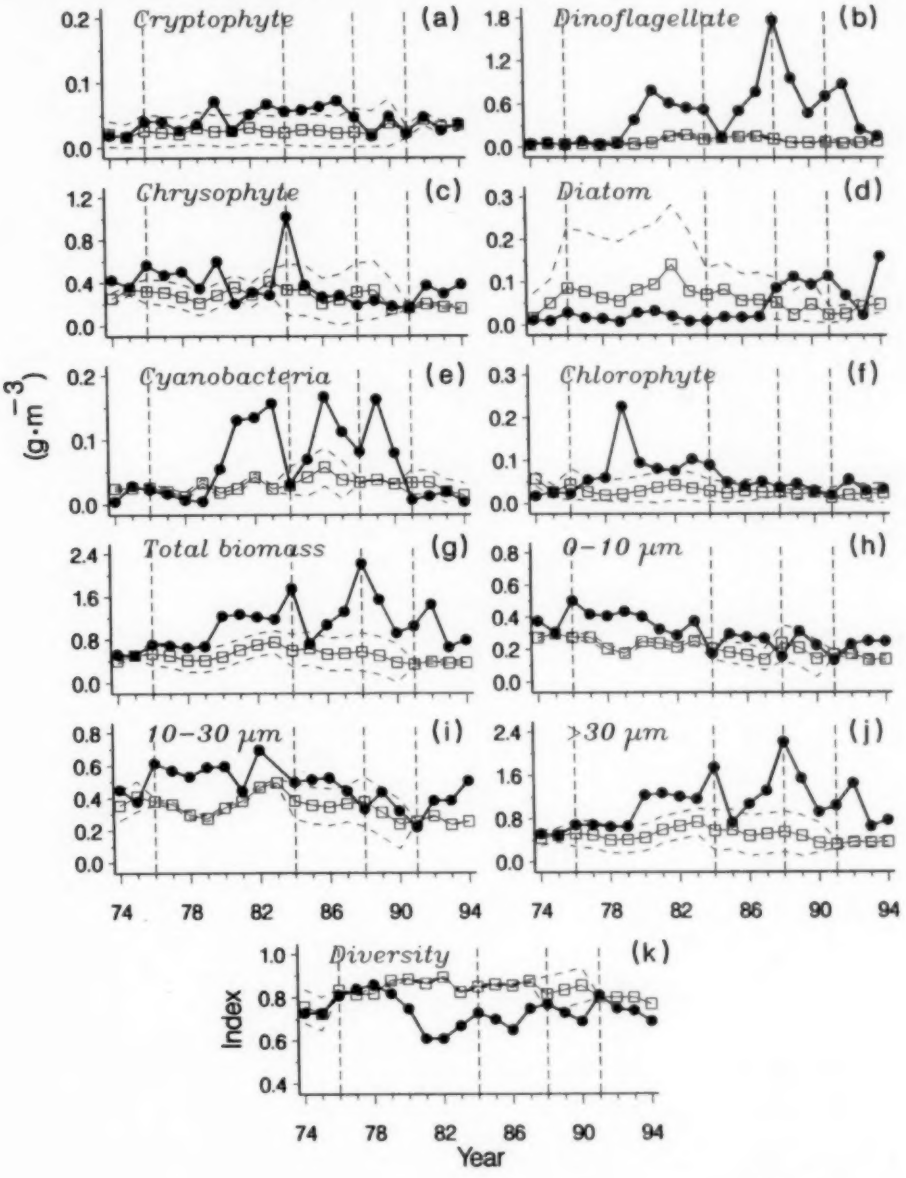
Fig. 2. Results of a nested ANOVA comparing epilimnetic Lake 223 with the epilimnetic mean of the reference lakes (1974–1994). ●, Lake 223; □, mean of reference lakes 224 and 239, with the horizontal broken lines showing ±2 SD. The vertical broken lines show the phases of the acidification and recovery of Lake 223 as indicated in Table 1. (*a*) Cryptophyte biomass; (*b*) dinoflagellate biomass; (*c*) chrysophyte biomass; (*d*) diatom biomass; (*e*) cyanobacteria biomass; (*f*) chlorophyte biomass; (*g*) total biomass; (*h*) size fraction of total biomass 0–10 μm; (*i*) size fraction of total biomass 10–30 μm; (*j*) size fraction of total biomass >30 μm; and (*k*) species diversity.



Results

The response of the Lake 223 phytoplankton community to acidification (1976–1983) and to the early phases of recovery (1984–1989) have been documented by Findlay and Kasian (1986, 1991).

During the acidification phase of Lake 223 (Table 1), major changes in the phytoplankton community occurred. Algal species dominance shifted initially from chrysophytes to a codominance by cyanobacteria, chlorophytes, and then to dinoflagellates below pH 5.6 (Fig. 1). Total phytoplankton biomass increased as pH decreased below pH 5.6, while

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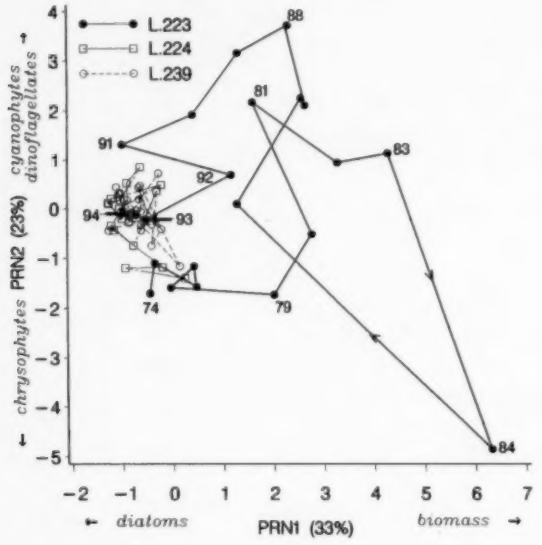
Fig. 3. The first two components from a principal components analysis of total phytoplankton biomass represented by each taxonomic group for lakes 223, 224, and 239. Axis 1 (PRN1) describes total biomass. The second axis (PRN2) distinguishes high cyanobacteria–dinoflagellate biomass from high chrysophyte biomass.



species diversity decreased (Figs. 2*g* and 2*k*). The size structure of the community was altered with phytoplankton species >30 µm representing a greater portion of the phytoplankton assemblage (Fig. 2*j*).

During the initial 7 years of recovery (phases 3 and 4, 1984–1990), there was little evidence the phytoplankton community had begun recovering (Findlay and Kasian 1991). Biomass remained elevated above pre-acidification estimates (Fig. 2*g*), and dinoflagellates continued to dominate the phytoplankton assemblage (Fig. 2*b*) with large populations of cyanobacteria present (Figs. 1 and 2*e*). Phytoplankton cells >30 µm continued to be over-represented in the assemblage. However, species diversity did increase but did not reach reference lakes levels (Fig. 2*k*).

In 1991 (phase 5) the pH of Lake 223 was increased to 6.1 and was maintained at that level through 1993 with pH increasing to 6.7 in 1994. Dinoflagellate dominance diminished during this period (Figs. 1 and 2*b*), and species dominance within the genus shifted from *Gymnodinium* sp. to *Peridinium limbatum*, *P. inconspicumm*, and *P. pusillum*. The year-to-year trend and overall mean for dinoflagellates in Lake 223, however, continued to be significantly different from those found in the reference lakes during this phase (Table 2). During the four recovery years when pH was above 6, chrysophytes became increasingly more abundant and dominated the phytoplankton assemblage several times during the 1992–1994 seasons (Fig. 1), mainly because of *Dinobryon bavaricum*, *D. sertularia*, *Chrysochromulina laurentiana*, and *Uroglena volvox*. These were species that were prominent prior to acidification. The year-to-year trend for chrysophycean biomass in Lake 223 from 1991 to 1994 was significantly different from the chrysophycean trend in the reference lakes, but the mean was not (Table 2). During phase 5 the trends and means for cyanobacterial, chlorophycean, and cryptophycean biomasses were not significantly different from the reference lakes, although cyanobacteria and chlorophytes previously had been (Fig. 2, Table 2). The year-to-year trend for diatom biomass in Lake 223 was significantly different from that of the reference system (Table 2), although diatom abundance was low.

The size structure of the phytoplankton community in Lake 223 during phase 5 was significantly different from pre-acidification structure and that of the reference lakes. This difference was due mainly to the continued abundance of species >30 µm (Fig. 2*j*, Table 2).

Total phytoplankton biomass remained elevated in 1991 and 1992 but declined to pre-acidification estimates in 1993–1994 (Fig. 2*g*). The trend over time and mean biomass in Lake 223 continued to be significantly different from those in the reference lakes during this phase (Table 2).

As pH increased above 6 in phase 5, species diversity initially reached levels attained during acidification and early recovery (Fig. 2*k*) but declined in successive years. The year-to-year trend for Lake 223 species diversity during this phase did not differ significantly from the reference lakes (Table 2), although the overall mean was significantly lower.

A principal components analysis of the seven taxonomic groups, accounting for 56% of the variance on the first two axes (Fig. 3), indicated that Lake 223 has become compositionally more similar to the reference lakes during 1991–1994 (Fig. 3). The first axis was a measure of total biomass. The second axis had high loadings for biomass of cyanobacteria, dinoflagellates, and chrysophytes.

During the two earliest phases of acidification (1984–1990), the fractions of N and P were relatively stable in Lake 223, although suspended N increased well beyond the levels recorded for the reference lakes in 1984 (Fig 4). The fraction of suspended C in Lake 223 from 1984 to 1990 was consistently higher than the reference lakes (Fig. 4*a*), coinciding with increased phytoplankton biomass (Fig. 2*g*). No significant changes were detected in phase 5 (1991–1994) in the concentrations of suspended C, P, or N, in the concentration of dissolved P or N, or in TP and TN (Fig. 4). Based on nutrient status indicators (Healey 1975), such as ratios of suspended C : chlorophyll, suspended C : suspended P, and suspended N : suspended P, the phytoplankton community in Lake 223 remains strongly P deficient (Fig. 5).

Discussion

The biological effects of acidification on Canadian lakes have been well documented and, in general, studies have elucidated similar results (Keith and Dillon 1989; Almer et al. 1974, 1978; Schindler et al. 1985, 1991; Findlay and Kasian 1986, 1990, 1991). However this statement is not true in regards to the biological recovery of these impacted lakes. Contrary to chemical recovery, biological recovery is inherently slower and much more ecosystem dependent (Havas 1989; Martin et al. 1986), i.e., biological recovery is dependent in part on chemical and physical recovery in addition to biotic recovery.

Havas (1989) discusses several terminologies that appear in the literature describing the response of systems from stresses such as acidification. Among these terms are recovery, revers-

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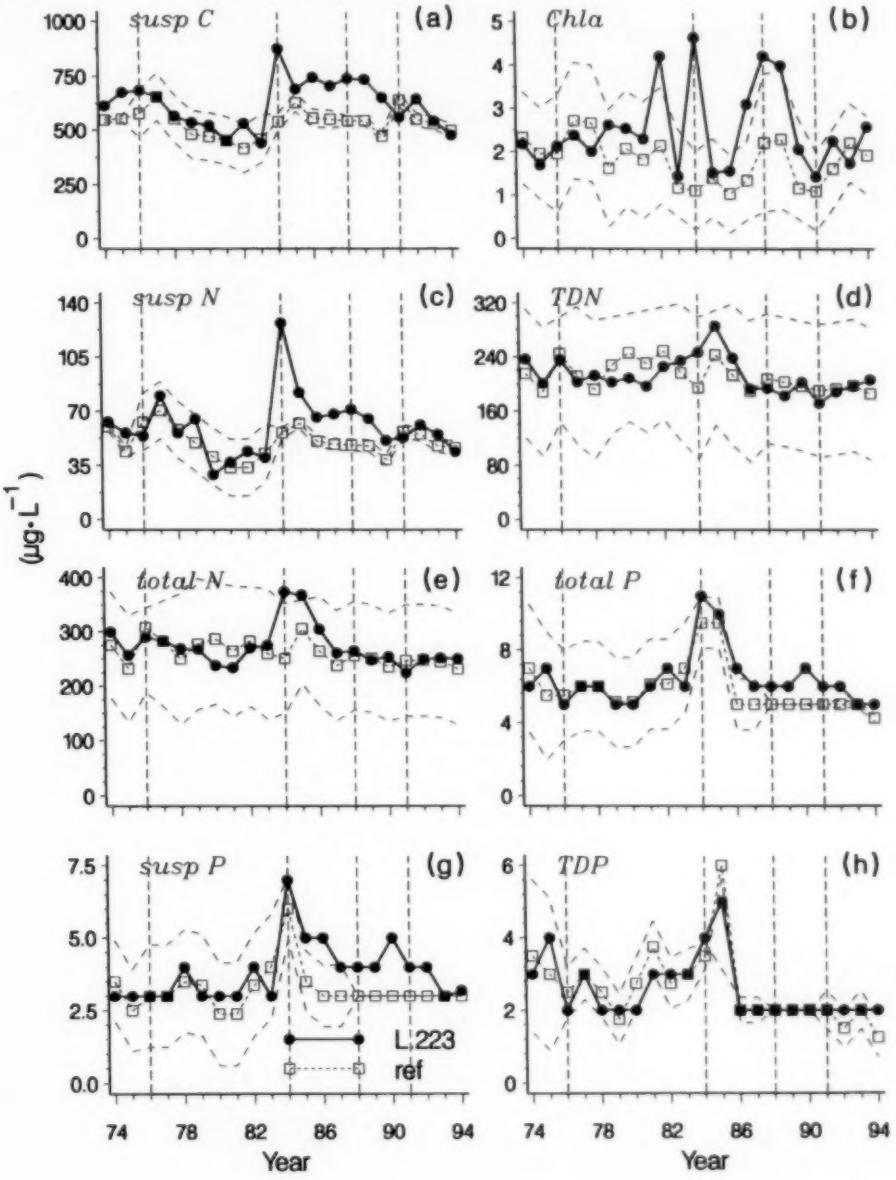
Fig. 4. Annual, ice-free, mean, epilimnetic nutrient concentrations ($\mu g \cdot L^{-1}$) for Lake 223 and the mean of reference lakes 224 and 239 for 1974–1994. ●, Lake 223; □, mean of reference lakes 224 and 239, with the horizontal broken lines showing ±2 SD. The vertical broken lines show the different stages of the acidification and recovery of Lake 223, as outlined in Table 1. (*a*) Suspended carbon; (*b*) chlorophyll; (*c*) suspended nitrogen; (*d*) total dissolved nitrogen; (*e*) total nitrogen; (*f*) total phosphorus; (*g*) suspended phosphorus; and (*h*) total dissolved phosphorus.



ibility, restoration, resiliency, and inertia. The problem with describing "recovery" is in fact defining what recovery is. Our definition of recovery for the Lake 223 phytoplankton community is based on its return to pre-acidification measures, with allowances for regional temporal changes, measured by communities in several reference lakes.

Nicholls et al. (1992), using three sets of survey data from lakes in the Sudbury area, concluded that increased numbers of phytoplankton taxa were indicative of recovery of these lakes from earlier acidification. Dixit et al. (1989), using chrysophyte scales as paleoindicators, concluded Swan Lake, Ontario, had recovered from previous acidification.

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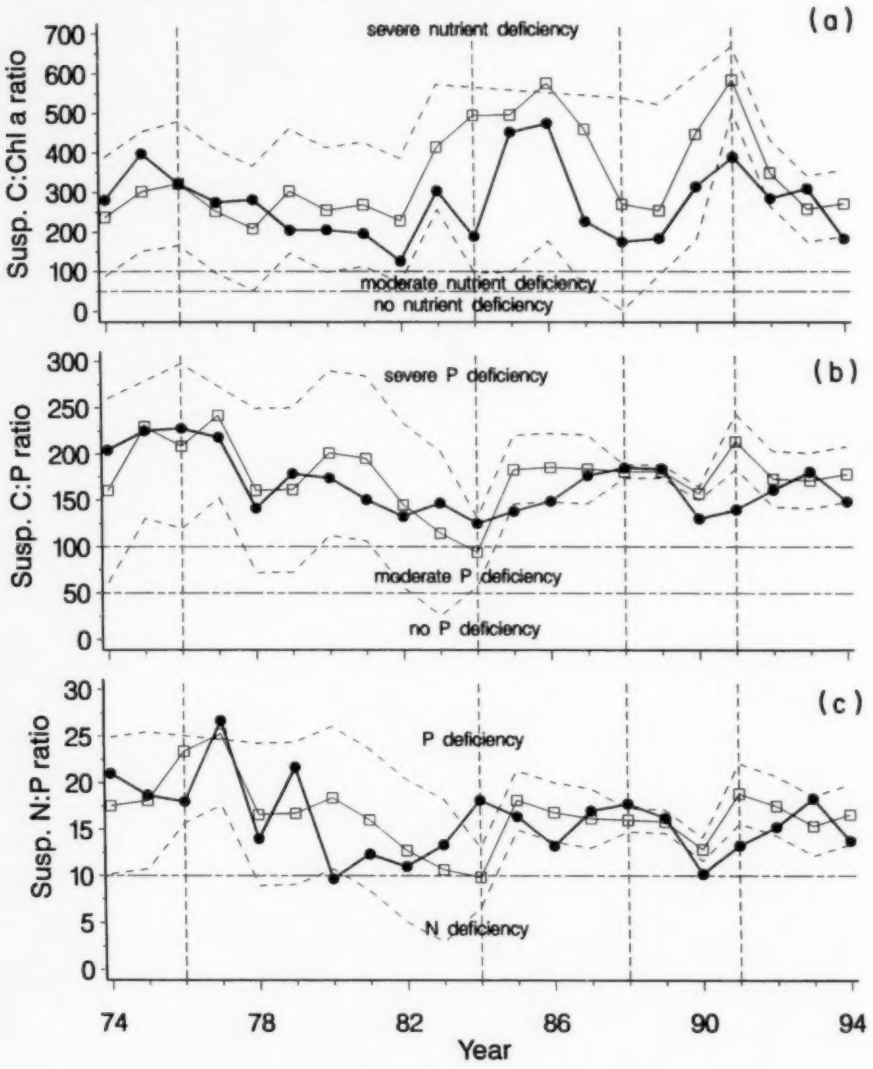
Fig. 5. Results of a nested ANOVA comparing ice-free, epilimnetic algal nutrient status indicators for Lake 223 with the mean of reference lakes 224 and 239 for 1974–1994. ●, Lake 223; □, mean of reference lakes 224 and 239, with the horizontal broken lines showing ±2 SD. The vertical broken lines show the different phases of the acidification and recovery of Lake 223 as outlined in Table 1. Values are the ratios (by weight) of (*a*) suspended carbon : chlorophyll; (*b*) suspended carbon : suspended phosphorus; (*c*) suspended nitrogen : suspended phosphorus.



Recovery of experimentally acidified Lake 223 was initiated in 1984. Because of the fast turnover time and resiliency of the phytoplankton, it was hypothesized that recovery of the phytoplankton community would be rapid, although pH dependent. As pH was incrementally increased from 5.1 to 5.8 in Lake 223, there was little indication that recovery had begun (Findlay and Kasian 1991). Phytoplankton biomass remained elevated and dinoflagellates continued to dominate even though previously dominant species were being introduced into Lake 223 from upstream Lake 224, a pristine reference lake. The only parameter indicating a progression toward recovery was species diversity. Findlay and Kasian (1991) hypothesized that recovery of the phytoplankton community in Lake 223 would occur 2–7 years after pH was elevated above 6.

Increasing the pH of Lake 223 to a level over 6 (phase 4) stimulated a strong recovery response within the phytoplankton community. Dinoflagellate biomass remained elevated initially but then decreased dramatically. Cyanobacteria biomass similarly declined to pre-acidification levels, while chryso-

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phyte biomass increased slightly as pH increased. Throughout the Lake 223 experiment, dinoflagellates have consistently been the major taxonomic group responding to acid stress, causing significant changes in other parameters such as biomass, diversity, and size structure. Some of these species have a selective advantage in that they are protected from adverse environments by a protective gelatinous sheath (*Gymnodinium* spp.). They are large, in many cases ornamented with spines, and very motile, making them less palatable. These taxa encyst under stressful conditions, remain dormant, and can return when environmental conditions are favorable. These ecological advantages may explain why dinoflagellates have such a wide pH tolerance and why their high abundances lag well into recovery.

Species diversity increased to pre-acidification levels as pH increased above 6. However, an obvious trend in species diversity occurred during the recovery phases. Diversity increased in the initial year that pH was elevated to a new threshold and decreased in the subsequent years when pH was maintained at a particular level. This may be explained by the continued presence, in the first year that pH is increased, of fringe acidic species existing at pH levels higher than their optimum tolerance levels. As pH is maintained over several years, many of these fringe species are outcompeted and diversity decreases.

The rate of recovery of the phytoplankton community in Lake 223 may have been accelerated by natural temporal events. The summers of 1992 and 1993 were the two wettest on record (K. Beaty, Department of Fisheries and Oceans, Winnipeg, Man., personal communication) resulting in increased flushing rates and potentially higher rates of introduction of species from upstream Lake 224 to Lake 223.

It is unreasonable to expect that the phytoplankton community in Lake 223 would return to its pre-acidification composition of 1974–1975. Kasian et al.[2] analyzed 15 reference systems with data records from 1 to 22 years showing that, in natural systems in the ELA, there is a degree of shift through time in natural phytoplankton communities that is associated with changing regional conditions. Therefore, we prefer to assess recovery relative to the range of conditions in natural lakes. But where acidification has affected whole regions, trends in different taxa are likely the best measure of recovery rate.

In the earliest phase of Lake 223 pH recovery, phytoplankton species diversity in Lake 223 was the only parameter to indicate an improvement. All other quantitative measures (biomass, species composition, and population size structure) indicated that Lake 223's phytoplankton assemblage remained similar to that of an acidified system and was distinctly different from the natural reference systems. However, once pH was increased over 6, many of the community parameters began to approach pre-acidification and reference lake estimates (Fig. 4), and it appears that the phytoplankton community in Lake 223 has recovered.

Acknowledgments

D. Cruikshank obtained the phytoplankton samples. The Freshwater Institute Water Chemistry Analytical Laboratory analyzed and summarized the chemical parameters.

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Evaluation of competition between age-0 yellow perch (*Perca flavescens*) and gizzard shad (*Dorosoma cepedianum*) in Oneida Lake, New York[1]

Edward F. Roseman, Edward L. Mills, John L. Forney, and Lars G. Rudstam

Abstract: Diet and growth of age-0 yellow perch (*Perca flavescens*) and age-0 gizzard shad (*Dorosoma cepedianum*) were compared during June-August 1990 and 1991. In both years, *Daphnia* dominated the zooplankton community in June and early July but disappeared in mid-July and early August. Age-0 yellow perch consumed primarily *Daphnia* and copepods until the *Daphnia* stocks were depleted then switched to benthic prey. Small age-0 gizzard shad primarily selected *Daphnia* and copepods while larger individuals consumed fewer zooplankton and more phytoplankton. Data from 1984 through 1993 indicated no direct effect of interspecific competition on growth rates of either species. The decrease in *Daphnia* populations in years with abundant age-0 gizzard shad occurs later in the year than in years with abundant age-0 yellow perch, and we suggest that this timing is crucial for the outcome of fish interactions. When *Daphnia* decline later in the season, larger age-0 yellow perch can switch to benthic prey and larger age-0 gizzard shad to phytoplankton; thus, the outcome of these interactions differs from the depression of age-0 bluegill (*Lepomis macrochirus*) by competition from age-0 shad reported from many reservoirs.

Résumé : On a comparé le régime alimentaire et la croissance de la perchaude (*Perca flavescens*) d'âge 0 et de l'alose à gésier (*Dorosoma cepedianum*) d'âge 0 au cours de la période juin–août de 1990 et de 1991. Au cours de ces deux années, *Daphnia* dominait la communauté zooplanctonique en juin et au début juillet, mais disparaissait à la mi-juillet et au début d'août. La perchaude d'âge 0 consommait principalement des *Daphnia* et des copépodes jusqu'à épuisement du stock de *Daphnia*, puis se nourrissait par la suite de proies benthiques. Les petites aloses à gésier d'âge 0 se nourrissaient surtout de *Daphnia* et de copépodes, tandis que les aloses plus grosses consommaient moins de zooplancton mais plus de phytoplancton. Selon des données obtenues de 1984 à 1993, la compétition interspécifique n'a aucun effet direct sur les taux de croissance des autres espèces. La baisse des populations de *Daphnia* au cours des années caractérisées par une abondance d'aloses à gésier d'âge 0 se produit plus tard dans l'année que pendant les années caractérisées par une abondance de perchaudes d'âge 0, ce qui, à notre avis, est très important pour les résultats des interactions entre les poissons. Lorsque les populations de *Daphnia* baissent plus tard dans la saison, les grosses perchaudes d'âge 0 peuvent commencer à se nourrir de proies benthiques, tandis que les grosses aloses à gésier d'âge 0 consommeront du phytoplancton; ainsi, les résultats de ces interactions sont différents de la baisse des populations de crapets à oreilles bleues (*Lepomis macrochirus*) d'âge 0 à la suite de la compétition exercée par les aloses à gésier d'âge 0, dont il a été fait état dans de nombreux réservoirs.
[Traduit par la Rédaction]

Introduction

The impact of zooplanktivorous age-0 gizzard shad (*Dorosoma cepedianum*) on zooplankton populations is well documented (Kutkuhn 1957; Cramer and Marzolf 1970; Barger and Kilambi 1980; Dettmers and Stein 1992; DeVries and Stein 1992; Shahady 1993). Age-0 gizzard shad can influence zooplankton populations directly by predation and indirectly by competitive herbivory (DeVries and Stein 1992). Because zooplankton is an important item in the diet of most larval fishes, the effect of age-0 gizzard shad on zooplankton dynamics could hamper growth of larval fish and, therefore, affect recruitment and productivity. The severity of this impact depends on how fish respond to declines in available food resources (Mills et al. 1987; Prout et al. 1990). In this paper we investigate the competitive interactions between age-0 gizzard shad and age-0 yellow perch (*Perca flavescens*) in Oneida Lake, New York.

Hanson and Leggett (1986) discuss conditions indicating competitive interactions in fish communities. Reduced growth of an indigenous species after the invasion of a new species and use of a limiting food resource by both species are evidence that competition occurs (Connell 1983). DeVries and Stein (1992) observed the effects of exploitative competition

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E.F. Roseman,[2] E.L. Mills, J.L. Forney, and L.G. Rudstam. Department of Natural Resources, Cornell University Biological Field Station, 900 Shackelton Point Road, Bridgeport, NY 13030, U.S.A.

[1] Contribution No. 180 of the Cornell Biological Field Station, Bridgeport, N.Y.

[2] Author to whom all correspondence should be sent to the following address. e-mail: rosemane@pilot.msu.edu
Present address: Department of Fisheries and Wildlife, 13 Natural Resources Building, Michigan State University, East Lansing, MI 48824–1222, U.S.A.

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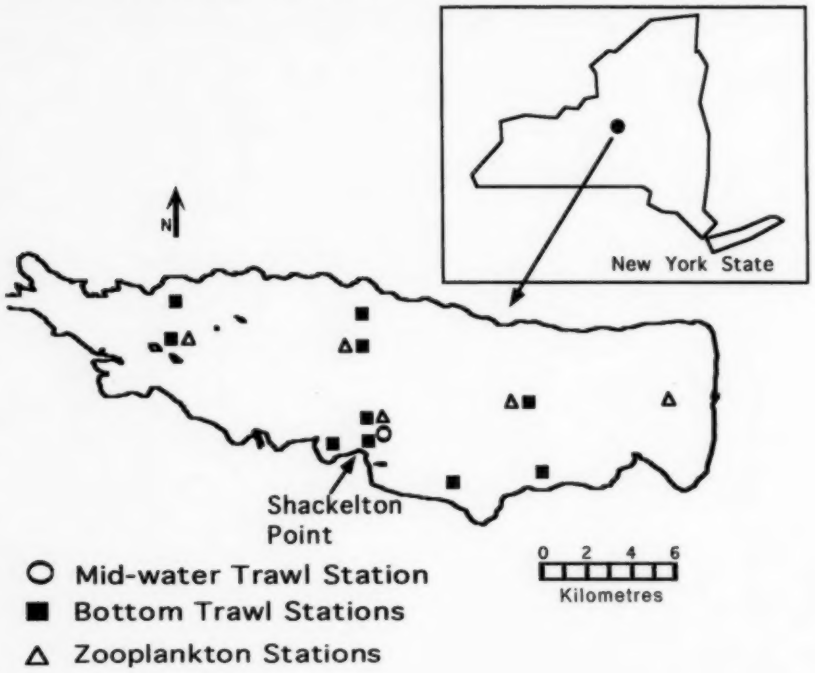
Fig. 1. Collection sites for age-0 yellow perch (bottom trawls), gizzard shad (mid-water trawl), and zooplankton in Oneida Lake, New York.



between larval gizzard shad and bluegill (*Lepomis macrochirus*) in Ohio reservoirs. In these systems, gizzard shad often drive crustacean zooplankton to low levels before bluegill begin exogenous feeding, leading to reduced bluegill growth. Reduced growth of bluegill potentially extends the period of vulnerability to gape-limited predators, thereby decreasing bluegill recruitment. Because gizzard shad, unlike bluegill, stay primarily offshore and grow faster than bluegill, gizzard shad cannot become a substitute prey for littoral largemouth bass (*Micropterus salmoides*). The result is an overabundance of gizzard shad and poor recruitment of bass. Studies of European perch (*Perca fluviatilis*) communities reveal that competitive effects of zooplanktivorous roach (*Rutilus rutilus*) force perch to seek alternative prey resulting in reduced growth and recruitment (Persson 1986; Persson and Greenberg 1990; Bergman and Greenberg 1994).

Oneida Lake is a naturally eutrophic and thermally well mixed warm-water percid lake (Mills et al. 1978) located on the Ontario plain of central New York State (Fig. 1). Trophic relationships in Oneida Lake are well documented, and during its recorded history, the biological structure of the lake has undergone substantial changes (see reviews in Mills et al. 1987; Mills and Forney 1988). In the 1960s and 1970s, walleye (*Stitzostedion vitreum vitreum*) and yellow perch functioned as a demographically simple predator–prey association (Forney 1980). During this period, planktivorous yellow perch were considered a keystone species and served as the primary link from secondary to higher trophic levels. Slow first-year growth of yellow perch resulting from the decline of *Daphnia* (Mills and Forney 1981) increased cannibalism by adult yellow perch (Tarby 1974) and prolonged the period of vulnerability to predation by walleye (Nielsen 1980). Consequently, cohorts of yellow perch that were exceptionally abundant in July and collapsed the *Daphnia* population experienced higher mortality than smaller cohorts (Forney 1980).

In 1984, large numbers of age-0 gizzard shad were first observed in Oneida Lake and young were present in widely varying numbers through 1993. Young gizzard shad generally occupy the pelagia of Oneida Lake. In this study, we compare the feeding of age-0 yellow perch and gizzard shad and examine the growth response of these fish to available food resources. Field studies were conducted in Oneida Lake in 1990 and 1991 when *Daphnia* populations collapsed and both yellow perch and gizzard shad were forced to seek alternate prey. Trends in abundance and growth of both fish species were compared with zooplankton using a larger historical data set.

Methods

Fish abundance and growth

Abundance of age-0 yellow perch was determined from the catch and area swept by an otter trawl with a 5.5-m footrope and a 6.4 mm mesh cod end. Samples were collected at 10 stations (Fig. 1) at weekly intervals from mid-July to mid-October. Catches generally declined exponentially and cohort abundance on 1 August was calculated by regressing the natural logarithms of the mean weekly catches on sampling date.

Age-0 gizzard shad were collected in Miller samplers at 24 randomly selected sites from June to August in 1984–1993 (Van De Valk et al. 1994). Surveys were repeated at 10-day intervals and successive estimates of larvae between 8 and 14 mm summed to

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Table 1. Historical data of yellow perch and gizzard shad abundance and growth in Oneida Lake, New York, for 1984–1993.

	Yellow perch				Gizzard shad					Zooplankton			
	Weight (g)				Weight (g)					Mean body size		*Daphnia* biomass	
Year	July	Oct.	SGR	No.·ha^{-1}	Aug.	Oct.	SGR	GSRI	GRD	June	Oct.	June	Oct.
1984	1.0	3.7	0.017	5030	1.2	16.4	0.045	355	182	0.74	0.49	109.2	23.6
1985	1.4	3.5	0.012	8360	0.3	4.5	0.044	170	184	0.86	0.44	110.4	0
1986	1.9	5.6	0.013	510	4.1	28.3	0.032	146	172	1.03	0.52	119.7	118.1
1987	1.7	3.1	0.007	960	1.7	13.6	0.035	941	166	1.01	0.35	86.1	0
1988	1.5	5.7	0.021	4000	10.3	20.9	0.022	350	163	0.85	0.58	443.3	21.4
1989	1.1	5.6	0.026	150	0.6	15.3	0.052	880	180	0.60	0.50	3.4	7.8
1990	1.3	3.7	0.017	2620	1.2	9.8	0.038	479	190	0.80	0.36	174.3	2.1
1991	2.0	5.1	0.015	900	3.9	12.0	0.018	768	166	0.83	0.43	174.3	14.9
1992	1.3	4.3	0.018	1780	0.3	3.7	0.039	673	183	1.09	1.17	275.2	193.3
1993	1.3	5.9	0.022	2820	0.3	9.2	0.056	208	202	1.17	0.72	179.5	0.0

Note: Values are the mean yellow perch wet weight (g) in early July and early October, specific growth rate (SGR), estimated abundance of age-0 yellow perch on 1 August (no.·ha^{-1}), mean gizzard shad wet weight (g) in early August and early October, specific growth rate (SGR), gizzard shad recruitment index (GSRI), day of year of peak recruitment of age-0 gizzard shad to 8–14 mm size class (GRD), mean zooplankton body size (mm), and *Daphnia* biomass (mg·L^{-1}).

approximate annual recruitment (GSRI) to the pelagic population. Examination of otoliths indicated larvae in the 8–14 mm class grew about 6 mm in 10 days (J.L. Forney, unpublished data); thus, the number of larvae on each sampling date represented the number recruited to the pelagic population between successive surveys. Yearling and older gizzard shad were seldom caught in trawls and variable mesh gill nets, which suggests few young survived over winter.

Additional samples of age-0 yellow perch and gizzard shad were collected weekly at the Shackelton Point study site (Fig. 1) in 1990 and 1991 to assess growth in weight and total length. Pelagic gizzard shad were collected using a mid-water trawl while yellow perch were collected with a bottom trawl. For yellow perch, average wet weight was calculated from the total catch for each sampling date while the average wet weight of gizzard shad was calculated for individual cohorts for each sampling date. Gizzard shad lengths were converted to weights using length–weight relations calculated from several samples of gizzard shad weighed individually in 1991 and 1992. Two equations were used: one for samples in which gizzard shad averaged less than 30 mm total length (TL) (ln weight (grams) = 3.121(ln TL) – 11.910) and one for larger fish (ln weight (grams) = 3.208(ln TL) – 12.370). Specific growth rates (SGR) were calculated with the equation $SGR = (\ln(w_t) - \ln(w_0))/d$, where w_t is the average weight of fish at time t; w_0 is the initial average weight; and d is the number of days between time t and time 0 (Ricker 1975).

Zooplankton community

Zooplankton abundance and composition were determined from weekly vertical hauls with a 0.5-m plankton net equipped with 153 mm mesh netting at five sites in Oneida Lake (Fig. 1). Additional zooplankton samples were collected concurrently and at the same depth as mid-water and bottom trawls for age-0 fish for use in calculating prey selection. Samples were preserved in sugar–formalin (Haney and Hall 1973). One to three 1-mL subsamples were withdrawn with a Hensen–Stemple pipette from a known volume of sample and counted using 20× projection onto an electronic touch screen interfaced with a microcomputer (Hambright and Fridman 1994). Additional subsamples were counted until at least 100 individual zooplankters had been enumerated. Zooplankton, except immature copepods (nauplii and copepodites), were identified to species (Pennak 1978; Balcer et al. 1984), counted, and measured. All nauplii and copepodites were classified as nauplii, counted, and measured. Functional groupings of microcrustacean and benthic invertebrate prey of fish were identified to taxonomic levels indicated in Tables 2 and 3.

Water temperatures were recorded with a YSI temperature recorder. We report the average rate of water warming (°C per day) from 1 May through 1 August using water temperature data from the Shackelton Point sampling site.

Diets of age-0 fish

Pelagic age-0 yellow perch and gizzard shad were collected using a mid-water trawl (3.6-m^2 mouth, 0.5 mm end cod mesh) towed at the surface for 5 min. Demersal yellow perch were collected using an otter trawl (5.5-m footrope, 6.4 mm end cod mesh) towed along the bottom for 10 min. Both gizzard shad and yellow perch were collected on the same date from the Shackelton Point site (Fig. 1). A random subsample of at least 50 fish of each species was anesthetized with tricaine methanesulfonate and preserved in unbuffered formalin for diet analysis.

Fish TL was measured to the nearest millimetre and wet weight to the nearest 0.001 g. Gizzard shad diets were compared for fish sorted into ≤20, 21–35, and >35 mm size groups. The size categories selected for gizzard shad were chosen to represent the different cohorts present during the protracted spawning period and to investigate the relation between oral gape and diet.

Stomach contents of individual fish were removed by dissection under a stereoscopic microscope. For gizzard shad, prey items were identified and counted either throughout the digestive tract (when the digestive tract was undifferentiated, typically <20 mm TL) or anterior to the pyloric caecum (when the digestive tract was well defined). For yellow perch, only prey items anterior to the pyloric caecum were enumerated. All prey in the stomach were identified according to the categories defined in Table 1, counted, and measured using 20× magnification projected onto a computer-interfaced electronic touch screen. We applied length – dry weight regressions (Cornell University Biological Field Station, unpublished data) to prey lengths to estimate the dry mass of prey items. When organisms were unmeasurable due to breakage or digestion, we used average values for the same prey item from fish examined from the same date.

Yellow perch and gizzard shad diets were characterized by frequency of occurrence, percent composition by biomass, and dry weight (milligrams) of prey per gram wet weight of fish for individual fish size categories. Prey electivity was estimated using the Manly–Chesson index, α_i, calculated as

$$\alpha_i = (r_i/n_i)/\sum_{i=1}^{m} r_i/n_i$$

where m is the number of prey sizes or species, r_i is the proportion of prey i ingested, and n_i is the proportion of prey i in the environment (Manly 1974; Chesson 1978; Lechowicz 1982). For m prey species in a sample, $\alpha \geq 1/m$ indicates positive selection of species i.

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Table 2. Stomach contents of age-0 yellow perch and gizzard shad collected June–August 1990 in Oneida Lake, New York.

Date	No. of fish	TL (mm)	Total biomass (μg)	% biomass of stomach content									
				SC	Cyc	Cal	Dap	N	Lep	Rot	Ch	Gam	Other
Yellow perch													
June 27	21	27.9	342		29	49	22	t					
July 2	40	34.4	286	t	1	75	23		1				
July 6	41	34.5	92	t	1	12	86	t	1				t
July 11	46	35.6	240		22	11	63		3				t
July 16	36	35.6	266	3	15	28	54						
July 20	20	44.1	962		39	45	16		t				
July 25	21	45.3	1077		9	16	75		t				
Aug. 3	20	52.4	2015		3	4	93		t				
Aug. 7	22	56.7	2796		1	32	2		t		4	58	4
Aug. 14	20	62.9	1052	t	1	12			8			51	28
Aug. 21	10	66.7	1545		t	4	t				25	48	23
Gizzard shad ≤20 mm													
June 27	11	12.5	7	6	73	t	13	8		t			
July 2	16	16.6	13	t	66		32	1		t			
July 6	20	16.2	13	t	95			4	t				
July 11	20	16.7	14	t	84			15					t
July 16	20	15.2	16	t	71	7		22					
July 20	20	17.6	18	2	46	6	28	18		t			
July 25	19	14.6	8		61	1	37			t			
Gizzard shad 20–35 mm													
July 2	3	22.0	26	7	65		28						
July 6	1	21.0	57		100								
July 11	20	27.2	34	12	36		37	15		t			
July 16	20	25.6	36	1	67	6	16	11					t
July 20	20	29.4	125	t	14	17	67	2					
July 25	20	23.4	59		74	2	23	1					
Aug. 3	18	22.2	16	1	36	12	49	2					
Aug. 7*	20	26.4	51	1	22	t	69	7					
Aug. 14*	20	30.1	33	2	30	50	13	5					
Aug. 21*	20	23.8	34	4	85	2	6	3		t			
Gizzard shad 35 mm													
July 20	20	38.6	178	t	14	24	61						
July 25	14	38.1	122		22	78							
Aug. 3*	20	45.4	166		5	7	88						
Aug. 7*	19	47.7	88		9	9	82						
Aug. 14*	20	41.8	58	2	42	t	53	2		t			
Aug. 21*	20	49.6	116	1	66	18	15	t		t			

Note: The percentages are based upon average biomass of prey ingested. An asterisk indicates the presence of algae in gizzard shad gut. TL, average fish total length; total biomass, average total biomass consumed per fish; SC, small cladocerans; Cyc, cyclopoid copepods; Cal, calanoid copepods; Dap, daphnids; N, nauplii; Lep, *Leptodora kindtii*; Rot, *Rotifer* spp.; Ch, chironomid larvae and pupae; Gam, *Gammarus* spp.; Ost, ostracods; Oth, unidentifiable dipterans, oligochaetes, tricopterans, ostracods, and other insects; t, trace (% biomass of stomach contents).

Diet overlap was assessed using Schoener's index α

$$\alpha = 1 - 0.5\left(\sum_{i=1}^{n} | p_{ij} - p_{ik} |\right)$$

where p_{ij} is the proportion of biomass of prey type i used by species j, and p_{ik} is the proportion of biomass of prey type i used by species k. This index gives a numeric value between 0 and 1. Values greater than 0.6 are considered biologically significant (Schoener 1970; Wallace 1981).

Analysis of historical data

Table 2 contains the historical data from 1984 to 1993 used to compare growth rates, abundances, and zooplankton community dynamics. Indices of age-0 yellow perch abundance are estimated from catches in bottom trawls. Values for gizzard shad recruitment index (GSRI) were calculated from catches of larval gizzard shad in Miller samplers (Van De Valk et al. 1994). Yellow perch hatch earlier and are generally 2–10 times heavier than young gizzard shad by early July in most years. Gizzard shad demonstrate a protracted spawning period in Oneida Lake, which introduces much variability to the average measurements of the age-0 cohort. We have listed average weights of age-0 gizzard shad in early August because more of the cohort would be recruited to our sampling gear than in July. We assessed the relationship between fish weight in early summer with their weight in October using Spearman's ranked correlation. We also used Spearman's ranked correlation to assess the relationships between indices of age-0 yellow perch abundance, gizzard shad abundance, and October *Daphnia* abundance. Simple linear regression was used to assess the relationship between gizzard shad recruitment date and size. The

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Mann–Whitney test was used to compare the timing of *Daphnia* collapse between years of high and low gizzard shad abundance. Statistical methods follow Snedecor and Cochran (1989) and Littell et al. (1991).

Results

Zooplankton, food habits, and growth of age-0 yellow perch and gizzard shad

1990

Waters warmed at a rate of 0.13°C · day^{-1} from 1 May through 1 August. The zooplankton community in 1990 was dominated by *Daphnia galeata mendotae* and *D. pulicaria* (30–64%), whereas calanoid copepods (*Skistodiaptomus oregonensis*, *Leptodiaptomus minutus*, and *Epischura lacustris*) and cyclopoid copepods (*Diacyclops thomasi bicuspidatus* and *Mesocyclops edax*) were generally the second most abundant groups (Fig. 2). Mean size of zooplankton was 0.86 mm in late June and declined to 0.64 mm by late August. The total zooplankton biomass was over 300 mg·L^{-1} in early July, declined throughout July, and remained below 150 mg·L^{-1} through August.

Yellow perch fed primarily on calanoid copepods in late June and the first week of July, switching to a diet of mostly *Daphnia* in mid-July (Table 2). Cyclopoid copepods, nauplii, and *Leptodora kindtii* were also consumed but in small amounts. Yellow perch became demersal between 11 and 20 July when the fish were approximately 35 mm TL. Yellow perch consumed primarily copepods and *Daphnia* until reaching a mean weight of 2.5 g in early August when their diet shifted to benthic macroinvertebrates. The shift to benthic invertebrates coincided with the depletion of the *Daphnia* stock (Table 2, Fig. 2). Stomach content (milligrams dry weight) consumed per gram of yellow perch was over 1.0 mg·g^{-1} in late June, then decreased abruptly to about 0.2 mg·g^{-1} in early July, and increased steadily to a peak near 1.6 mg·g^{-1} in early August before declining again (Fig. 2).

Gizzard shad ≤20 mm fed primarily on cyclopoid copepods throughout the summer, which accounted for 46–95% of the biomass in their stomachs. Total zooplankton prey biomass per gut never exceeded 18 mg for gizzard shad ≤20 mm (Table 2). Larger gizzard shad (20–35 mm) consumed mainly cyclopoid copepods (14–100%) and *Daphnia* (6–69%) throughout the summer with the exception of 14 August when calanoid copepods were the dominant prey. Total biomass of zooplankton prey per gut peaked for 20–35 mm gizzard shad at 125 mg on 20 July and declined through the rest of the study (Table 2). Algae and other organic material (not quantified in this study) were prevalent in larger (>30 mm) gizzard shad of this size class in early August. Gizzard shad >35 mm fed primarily on *Daphnia* and copepods (96–100% of zooplankton biomass) though small cladocerans, rotifers, and nauplii increased in diets in August (Table 2). Total prey biomass per gut for gizzard shad >35 mm was highest on 20 July and declined subsequently (Table 2). For all size classes combined, zooplankton prey biomass of age-0 gizzard shad decreased from approximately 1.5 to 0.2 mg·g^{-1} from June through July (Fig. 2).

Diet overlap was strongest (α = 0.4–0.8) between yellow perch and gizzard shad >20 mm in early July through early August when both species of fish were consuming *Daphnia* (Fig. 2). Electivity index values indicate that yellow perch preferred copepods and small cladocerans when smaller than 35 mm and preferred *Daphnia* when larger than 35 mm. Gizzard shad ≤35 mm selected for small cladocerans and cyclopoid copepods. *Daphnia* were selected by larger gizzard shad in August (Fig. 3).

Yellow perch average body weight increased steadily from late June through early August (Fig. 2), and the weight gain was matched by an increase in total prey biomass per gut and prey biomass per gram of fish over the same time period (Fig. 2, Table 2). The decline in yellow perch growth rate between 7 and 14 August (Fig. 4) coincided with a sharp

Fig. 2. (A) Prey biomass (mg) per gram of age-0 fish estimated from stomach contents of yellow perch and gizzard shad collected from Oneida Lake, June–August 1990. (B) Schoener's index of diet overlap between age-0 yellow perch and gizzard shad from Oneida Lake, 1990. (C) Increase in weight of age-0 yellow perch and gizzard shad in Oneida Lake, June–August 1990. (D) Zooplankton composition based on biomass averaged for five sampling stations in Oneida Lake, June–August 1990. Ordinate scale varies.

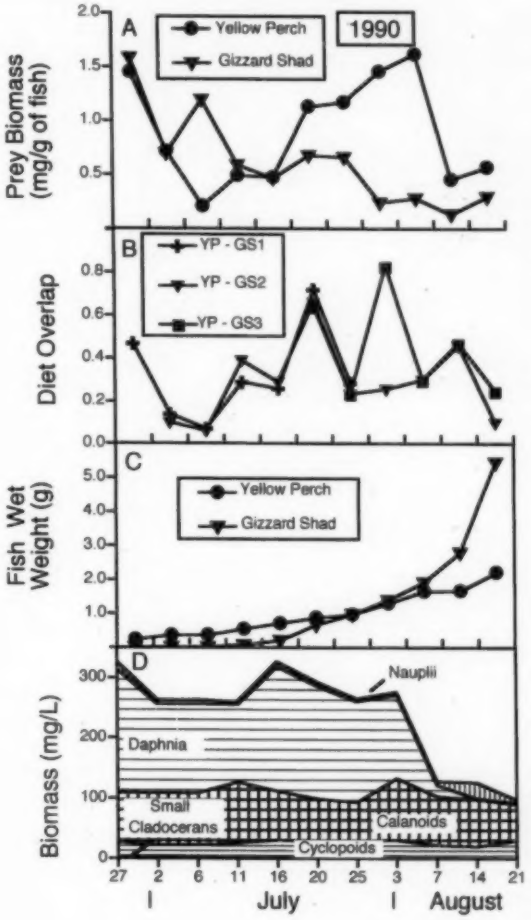

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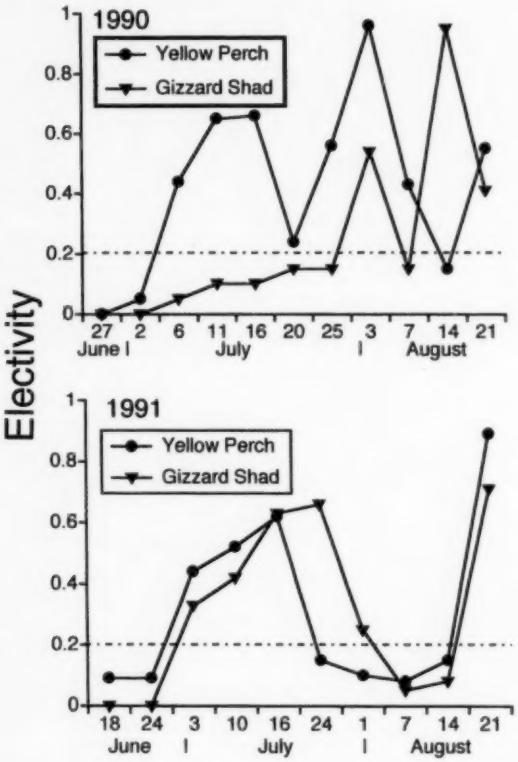
Fig. 3. Manly–Chesson electivity index values for *Daphnia* consumed by age-0 yellow perch and gizzard shad in Oneida Lake, June–August 1990 (top) and 1991 (bottom). Values for gizzard shad are for all size-classes combined. Broken lines indicate value of neutral selection.

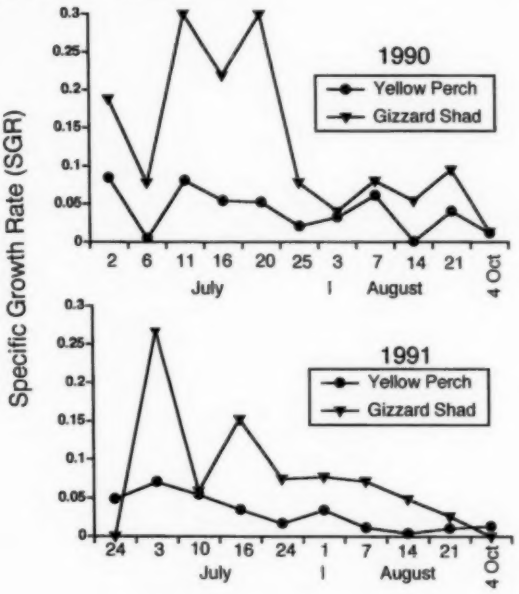


decrease in total prey biomass per gut (Fig. 2, Table 2). Specific growth rate of age-0 gizzard shad was greatest for small fish when zooplankton, primarily *Daphnia*, were the major component of the diet. Gizzard shad SGR declined markedly in late July and generally matched or exceeded yellow perch in August and early October (Fig. 4) as detritus and algae began to dominate their diets. However, SGRs remained higher for gizzard shad than for yellow perch throughout the year, resulting in gizzard shad weights nearly three times greater than for yellow perch at the end of the summer.

1991

Waters warmed at a rate of 0.14°C·day^{-1} from 1 May through 1 August. *Daphnia* were abundant in the zooplankton community until mid-July when the population began to diminish and calanoid copepods and nauplii gained in relative abundance (Fig. 4). Mean size of crustacean zooplankton was 1.1 mm in late June and declined to 0.4 mm by 21 August. The total zooplankton biomass reached nearly 300 mg·L^{-1} by late June and declined markedly throughout the summer to a low of 92 mg·L^{-1} by early August. Zooplankton biomass was dominated by *Daphnia* (35–70%) in late June and early July and by calanoid copepods in late July and August.

Fig. 4. Specific growth rates (SGR) for age-0 yellow perch and gizzard shad collected from Oneida Lake, June–August 1990 (top) and 1991 (bottom).



Yellow perch ate primarily copepods and *Daphnia* (99% of prey biomass) between 18 and 24 June, switching to mainly *Daphnia* in early July. When *Daphnia* stocks were depleted in late July, yellow perch shifted to copepods and benthic prey (Table 3). Yellow perch became demersal between 18 and 24 June when fish averaged 35 mm TL. Total prey biomass (milligrams) per yellow perch gut increased through June and July to a peak of 5.0 mg in mid-July and then declined to about 2.0 mg for the remainder of the study (Table 3). Prey biomass (milligrams dry weight) per gram of yellow perch peaked in mid-July (at the same time as the total biomass per gut peaked) and then declined to just less than 1.0 mg·g^{-1} (Fig. 5).

Gizzard shad ≤20 mm consumed primarily cyclopoid copepods (41–79% of prey biomass) until the size-class disappeared in mid-July, while 20–35 mm gizzard shad fed heavily on *Daphnia* through mid-July (40–94% of the prey biomass) (Table 3). *Daphnia* generally dominated the diet of the largest gizzard shad (>35 mm), but the total zooplankton biomass per gizzard shad gut declined dramatically after mid-July when the gizzard shad were approximately 1.2 g (50 mm TL) and the lake population became nondetectable (Fig. 5). The biomass of zooplankton prey (milligrams) per gram of gizzard shad >35 mm declined to near zero after mid-July. For all size-classes combined, prey biomass of age-0 gizzard shad declined from near 1.5 mg·g^{-1} in late June to less than 0.2 mg·g^{-1} in August (Fig. 5).

Diet overlap was significant ($\alpha > 0.6$) between yellow perch and gizzard shad 20–35 mm in early July when both species of fish were feeding heavily on *Daphnia* (Table 3, Fig. 5). Diet overlap values declined sharply after mid-July when *Daphnia* stocks diminished and yellow perch began feeding on benthic macroinvertebrates (Fig. 5). Manly–Chesson

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Table 3. Stomach contents of age-0 yellow perch and gizzard shad collected June–August 1991 in Oneida Lake, New York.

Date	No. of fish	TL (mm)	Total biomass (mg)	% biomass of stomach content									
				SC	Cyc	Cal	Dap	N	Lep	Rot	Ch	Gam	Other
Yellow perch													
June 18	20	31.5	148	t	31	30	38	t					
June 24	40	35.8	937	t	11	84	5		t				
July 3	20	45.5	567		8	22	70						
July 10	20	49.5	695		2	26	58	t			2	8	3
July 16	20	53.9	5007	t	t	60	39		t				
July 24	20	56.8	1293	t	10	13	t	t	t		4	68	4
Aug. 1	20	58.9	2300	t	6	5	t	t	t		13	73	2
Aug. 7	20	64.1	1837	t	9	2	1				5	79	5
Aug. 14	18	63.4	26	28		28	4	1		t		462	2
Aug. 21	18	64.7	1177		10	9	20		t		15	38	8
Gizzard shad ≤20 mm													
June 18	20	15.6	18		67	15	4	14					
June 24	20	16.3	26	5	71	2	9	13		t			
July 3	20	13.1	5	10	52	t		38					
July 10	20	17.5	26	2	41	1	40	16					
July 16	16	17.8	9		79	1		20					
Gizzard shad 20–35 mm													
June 24	20	25.7	128	t	24	2	72	2					
July 3	20	26.4	40		12		80	6	2	t			
July 10	20	27.2	86	t	13	1	83	2					
July 16	19	29.2	104		1		94	t	4				
July 24*	20	26.2	32	5	47		44	4					
Aug. 1*	20	31.4	17	12	5	51	16	16		t			
Gizzard shad >35 mm													
July 3	19	36.8	200		10	27	63	t					
July 10	20	45.3	341	t	2	34	64	t					
July 16*	20	49.4	356		5	17	77		1				
July 24*	20	62.1	84	t	32	24	39	4					
Aug. 1*	20	51.7	12	4	18	t	70	8		t			
Aug. 7*	20	45.4	11	20	52	t	20	7		t			
Aug. 14*	20	67.6	5	67				33		t			
Aug. 21*	20	69.4	13	18			56	5	22	t			

Note: The percentages are based upon average biomass of prey ingested. An asterisk indicates the presence of algae in gizzard shad gut. TL, average fish total length; total biomass, average total biomass consumed per fish; SC, small cladocerans; Cyc, cyclopoid copepods; Cal, calanoid copepods; Dap, daphnids; N, nauplii; Lep, *Leptodora kindtii*; Rot, *Rotifer* spp.; Ch, chironomid larvae and pupae; Gam, *Gammarus* spp.; Ost, ostracods; Oth, unidentifiable dipterans, oligochaetes, tricopterans, ostracods, and other insects; t, trace (<1% biomass of stomach contents).

electivity index values for age-0 yellow perch indicate strong selection for *Daphnia* throughout July and again in late August (Fig. 3). Electivity values for gizzard shad ≤20 mm showed positive selection for small cladocerans, cyclopoid and calanoid copepods, and nauplii. Larger gizzard shad exhibited positive selection for cyclopoid and calanoid copepods and *Daphnia*. Electivity values for all size-classes of age-0 gizzard shad combined indicate strong selection for *Daphnia* throughout July and again in late August (Fig. 3).

Specific growth rates of age-0 yellow perch declined from June through October and were generally lower compared with age-0 gizzard shad. Even though gizzard shad were smaller than yellow perch in June and early July, gizzard shad grew much faster than yellow perch after mid-July, reaching nearly 8.0 g by the end of August (Fig. 5).

Historical changes in abundance and growth of age-0 gizzard shad and yellow perch

Weights, indices of abundance, and SGRs for age-0 yellow perch and gizzard shad from late July to mid-October are summarized in Table 2. Gizzard shad weights exceeded those of yellow perch by mid-October in all years except 1992. Fall weights of yellow perch were not correlated with their weights in early July (Spearman's rank correlation coefficient (r_s) = 0.073, $N = 10$, $p = 0.94$); however, fall weights of gizzard shad did show a significant positive correlation with gizzard shad weights in early August ($r_s = 0.708$, $N = 9$, $p = 0.04$). For all years, SGRs were higher for gizzard shad than yellow perch.

Indices of age-0 yellow perch and gizzard shad abundance were highly variable among years (Table 2). Relationships between fish abundance and biomass of *Daphnia* in the fall indicate that age-0 yellow perch abundance is inversely but weakly related to *Daphnia* biomass ($r_s = -0.39$, $N = 10$, $p = 0.24$), while there was no relationship between GSRI and *Daphnia* ($r_s = -0.05$, $N = 10$, $p = 0.87$). Negative relationships were observed between October yellow perch weight and yellow perch abundance on 1 August ($r_s = -0.218$, $N = 10$, $p = 0.51$), October gizzard shad weight and gizzard shad

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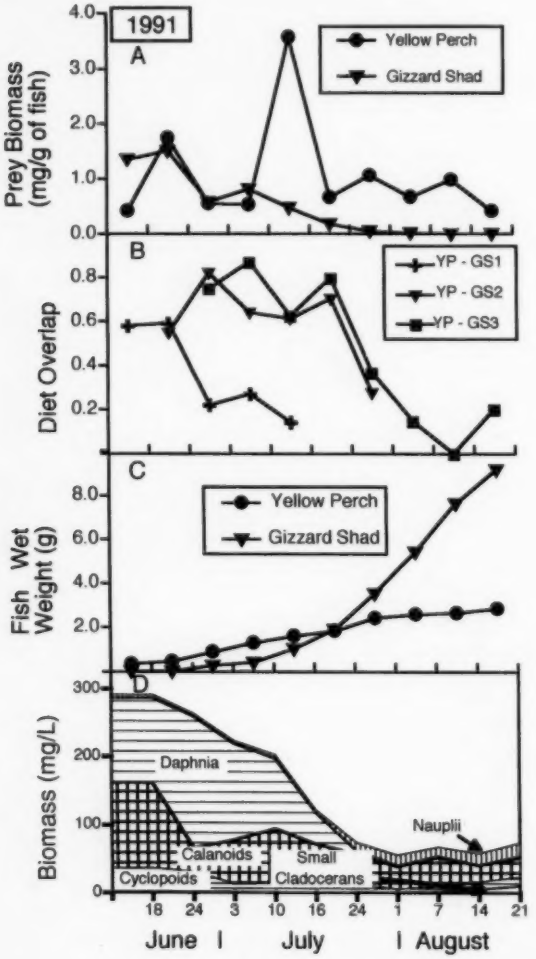
Fig. 5. (A) Prey biomass (mg) per gram of age-0 fish estimated from stomach contents of yellow perch and gizzard shad collected from Oneida Lake, June–August 1991. (B) Schoener's index of diet overlap between age-0 yellow perch and gizzard shad from Oneida Lake, 1991. (C) Increase in weight of age-0 yellow perch and gizzard shad in Oneida Lake, June–August 1991. (D) Zooplankton composition based on biomass averaged for five sampling stations in Oneida Lake, June–August 1991. Ordinate scale varies.



abundance (GSRI) ($r_s = -0.333$, $N = 10$, $p = 0.35$), October yellow perch weight and GSRI ($r_s = -0.309$, $N = 10$, $p = 0.35$), and fall gizzard shad weight and yellow perch abundance ($r_s = -0.217$, $N = 9$, $p = 0.54$), although none was significant.

Daphnia stocks declined between 29 July and 2 September in the years 1984–1993 (2 September 1985, 29 July 1987, 19 August 1989, 26 August 1990, and 6 August 1991), whereas similar declines in the years 1964–1983 attributed to abundant age-0 yellow perch were earlier, between 11 July and 5 August (11 July 1965, 22 July 1971, 15 July 1975, 5 August 1977, and 15 July 1981) (Cornell University Biological Field Station, unpublished data). The depletion of *Daphnia* stocks occurred significantly later in years after 1984 when gizzard shad were more abundant than in previous years (Mann–Whitney U statistic, $W = 16$, $\alpha = 0.0212$).

Discussion

Larval gizzard shad are considered visual particulate feeders until they switch to a pump-filter feeding mode at sizes >25 mm TL when they are capable of feeding on zooplankton, phytoplankton, and detritus (Drenner et al. 1982*a*, 1982*b*). In this study, gizzard shad fed on zooplankton, particularly *Daphnia*, until densities of these organisms became low and young gizzard shad started to feed on algae and detritus. In this study, gizzard shad >35 mm had lower zooplankton prey biomass (milligrams per gram fish) per gut than the smaller gizzard shad and guts of larger gizzard shad contained copious amounts of phytoplankton. Cramer and Marzolf (1970) observed patterns in planktivory similar to those found in this study when they examined the diets of these size-classes of age-0 gizzard shad in Tuttle Creek Reservoir, Kansas; crustacean zooplankton were the dominant prey of the smallest age-0 gizzard shad and phytoplankton became increasingly important as the fish exceeded 20 mm. Kutkuhn (1957) observed that zooplankton account for approximately 25% of the plankton ingested by age-0 gizzard shad 24–82 mm TL in North Twin Lake, Iowa.

Compared with age-0 yellow perch, young gizzard shad biomass in Oneida Lake generally peaks later in August, and if *Daphnia* disappear at this time, gizzard shad become omnivorous and feed on small zooplankton and phytoplankton. The disappearance of *Daphnia* has been shown to depress the growth of both age-0 yellow perch and age-0 white perch (*Morone americana*) (Mills and Forney 1981; Prout et al. 1990), but the disappearance of *Daphnia* in this study did not depress the growth in weight of young gizzard shad. In fact, weight of young gizzard shad continued to increase fast as these fish switched to feeding on other zooplankton and algae. The decrease in the amount of large-bodied crustacean zooplankton and the increasing prevalence of algae in diets as age-0 gizzard shad grow in Oneida Lake suggests that these fish switch from visual particulate feeding to pump-filter feeding. The ability of these fish to shift feeding mode allows young gizzard shad to maintain their growth when supplies of certain planktonic food organisms become low.

The years 1985, 1992, and 1993 were years of late recruitment for gizzard shad, which is reflected in their low summer weights (Table 2). We regressed time of peak gizzard shad recruitment and gizzard shad weights from 1984 to 1993. Day of peak recruitment to the 8–14 mm size-class accounts for 53% of the variability in weight on 1 August and 26% of the variability in October mean weights. The timing of peak recruitment of gizzard shad to the 8–14 mm size-class may also be reflected in the level of diet overlap between age-0 gizzard shad and yellow perch in early summer. Figures 2 and 5 (panel B) reveal that diet overlap was higher in June and early July of 1991 than 1990. Because small gizzard shad (<20 mm TL) diets contain few of the same prey items as age-0 yellow perch from the same dates (Tables 2 and 3), we would expect diet overlap to be low during these periods as was observed in 1990. Gizzard shad spawned earlier and grew faster in 1991 thus allowing for greater diet overlap earlier in the summer.

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Mean weight of age-0 gizzard shad in Oneida Lake in October exceeded age-0 yellow perch in all years except 1992 (Table 2). Rapid first-year growth of gizzard shad is common throughout its range. Lengths of age-0 gizzard shad in fall exceeded 160 mm in Smith Mountain Lake, Virginia (Tisa and Ney 1991), 200 mm in western Lake Erie (Bodola 1965), and 220 mm in Lake Newnan, Florida (Berry 1957). Such rapid growth is attributed to the opportunistic omnivorous feeding strategy adopted by gizzard shad. In contrast, mean weight of age-0 yellow perch in Oneida Lake was lower compared with gizzard shad in all years except 1992. Earlier studies have shown that growth of yellow perch in Oneida Lake is dependent on the availability of *Daphnia*, and yellow perch growth is slowed when *Daphnia* collapse and perch are forced to seek alternate prey.

Gizzard shad were abundant in Oneida Lake between 1984 and 1993. During this period, *Daphnia* declined to low levels ($<1\ \mu g \cdot L^{-1}$) in 5 of the 10 years. Because yellow perch growth rates have been shown to be negatively affected by low *Daphnia* abundance, we expected to see a negative effect of gizzard shad on age-0 yellow perch growth rates. This was not observed. We suggest that this is due to the timing of the *Daphnia* decline to $<0.5 \cdot L^{-1}$ that we attribute to gizzard shad.

In a series of papers, Stein, DeVries, and Dettmers have shown a strong negative effect of age-0 gizzard shad on bluegill recruitment in Ohio reservoirs (DeVries and Stein 1992; Dettmers et al. 1995; Stein et al. 1995). They attribute this effect to exploitative competition for zooplankton. A similar strong negative effect of age-0 shad on age-0 yellow perch was not observed in Oneida Lake even though both species compete for zooplankton. We suggest this is due to two key differences between the shad–bluegill and shad – yellow perch interactions: (*i*) yellow perch appear in the limnetic zone before shad and are not affected by competition for zooplankton until later in the summer when they already reached a relatively large size, whereas bluegill are affected in the early larval stage, and (*ii*) when zooplankton are depleted in late summer by gizzard shad, yellow perch, because of their large size, can switch to benthic prey and shad to phytoplankton without detectable changes in growth rates. Why yellow perch have not attained high enough densities in Oneida Lake since shad became abundant (1984) to deplete *Daphnia* stocks (as observed in the 1970s; Mills and Forney 1988) remains to be understood. We believe this is due to the result of the three-way interaction between shad, yellow perch, and their shared predator, walleye. Although shad potentially compete with yellow perch, they also serve as an alternate prey for walleye and may allow the buildup of large walleye populations, thereby increasing predation on the earlier life stages of yellow perch. Such a possibility is currently being explored through analysis of the 40-year data set of fish population dynamics from Oneida Lake.

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Relationship between sediment and plankton isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) and primary productivity in Florida lakes[1]

Binhe Gu, Claire L. Schelske, and Mark Brenner

Abstract: Surface sediment and planktonic organic matter from Florida lakes were analyzed for $\delta^{13}C$ and $\delta^{15}N$ to study the relationship between stable isotope ratios and lake productivity inferred from measurements of Chl *a*. Stepwise regression indicates that sediment $\delta^{13}C$ is a function of primary productivity, which is also suggested by a strong correlation between water column Chl *a* and planktonic $\delta^{13}C$ ($r = 0.82$, $P = 0.002$). However, Chl *a* explains only 38% of the variability of sediment $\delta^{13}C$. This weak correlation is mainly attributed to the presence of nonplanktonic organic matter bearing a distinct isotopic signature in the lake sediments. Sediment and planktonic $\delta^{13}C$ are also significantly correlated with water-column DIC, $CO_{2(aq)}$, $\delta^{13}C$ of DIC, TN, and TP. Sediment and planktonic $\delta^{15}N$ increase from oligotrophic to eutrophic lakes but decrease in hypereutrophic lakes. The nonlinearity of $\delta^{15}N$ across the trophic gradient of study lakes may be a consequence of the relative importance of cyanobacterial N_2 fixation, but this hypothesis needs further testing.

Résumé : On a dosé le $\delta^{13}C$ et le $\delta^{15}N$ dans des sédiments de surface et de la matière organique planctonique prélevés dans des lacs de Floride, en vue d'étudier la relation entre les rapports d'isotopes stables et la productivité des lacs déduite à partir de mesures de la chlorophylle *a*. Selon les résultats d'une analyse de régression par degrés, le $\delta^{13}C$ dans les sédiments est une fonction de la productivité primaire, ce que laisse aussi supposer l'existence d'une étroite corrélation entre la chlorophylle *a* et le $\delta^{13}C$ dans la colonne d'eau ($r = 0{,}82$, $P = 0{,}002$). Toutefois, la chlorophylle *a* permet de rendre compte de 38% seulement de la variabilité du $\delta^{13}C$ dans les sédiments. Cette faible corrélation a surtout été attribuée à la présence de matière organique non planctonique portant une signature isotopique distincte dans les sédiments lacustres. Il existe aussi une corrélation significative entre le $\delta^{13}C$ dans les sédiments et dans le plancton et la teneur en carbone inorganique dissous dans la colonne d'eau, la teneur en $CO_{2(aq)}$, la teneur en $\delta^{13}C$ du carbone inorganique dissous, l'azote total et le phosphore total. Le $\delta^{15}N$ dans les sédiments et dans le plancton augmente dans les lacs allant d'oligotrophes à eutrophes, mais diminue dans les lacs hypereutrophes. La non-linéarité du $\delta^{15}N$ dans le gradient trophique des lacs étudiés est peut-être la conséquence de l'importance relative de la fixation de N_2 par les cyanobactéries, mais il y aurait lieu de poursuivre la vérification de cette hypothèse.
[Traduit par la Rédaction]

Introduction

Stable carbon isotope ratios ($\delta^{13}C$) of sediment bulk organic matter have been used to explain variations in paleoproductivity (Calvert et al. 1992; Schelske and Hodell 1991, 1995; Shemesh et al. 1993) and atmospheric carbon dioxide levels (Arthur et al. 1985; Jasper and Hayes 1990). Stable nitrogen isotopes ($\delta^{15}N$) of sediment organic matter have also been used to infer paleoproductivity (Calvert et al. 1992; Shemesh et al. 1993), nitrate concentration (Altabet and Francois 1994), and nitrogen fixation (Talbot and Johannessen 1992). A number of variables can affect $\delta^{13}C$ of planktonic organic matter in aquatic systems. These variables include, but are not limited to, dissolved free carbon dioxide ($CO_{2(aq)}$) concentration, species composition, algal growth rate, and differential use of $CO_{2(aq)}$ and HCO_3^- (Fry and Sherr 1984; Goericke et al. 1994). $\delta^{15}N$ of primary producers changes with substrate level and growth rate (Mariotti et al. 1984; Wada and Hattori 1978) and with N_2 fixation (Hoering and Ford 1960). $\delta^{15}N$ of cyanobacteria decreases with increasing N_2 fixation rate in freshwater ecosystems (Estep and Vigg 1985; Gu and Alexander 1993).

Research on the use of sediment isotope records as ecological and environmental proxies has focused principally on marine systems (see reviews by Fry and Sherr 1984 and Owens 1987). There are only a few studies on freshwater lakes (e.g., Hollander and McKenzie 1991; Schelske and Hodell 1991, 1995). In most studies, only $\delta^{13}C$ was reported; $\delta^{15}N$ is rarely measured, although it is potentially an effective environmental research tool (Owens 1987). In addition, most work has been carried out on sediment cores from a single ecosystem. To our knowledge there are no studies that have used stable isotope data from sediment organic matter to examine trophic relationships among lakes.

It is now commonplace to assess the trophic state of lakes using data for TP, TN, Secchi depth, and Chl *a* from the water column (Carlson 1977; Kratzer and Brezonik 1981). The standing crop of phytoplankton measured as Chl *a* is dependent

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B. Gu,[2] C.L. Schelske, and M. Brenner. Department of Fisheries and Aquatic Sciences, University of Florida, 7922 NW 71st Street, Gainesville, FL 32653, U.S.A.

[1] Journal series No. R–04727 of the Florida Agricultural Experiment Station.

[2] Author to whom all correspondence should be addressed. e-mail: binhe@nervm.nerdc.ufl.edu

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on the concentration of TP, TN, or both in lakes. It is generally assumed that standing crops of Chl *a* are dependent on primary productivity in lakes (Beaver and Crisman 1991; Smith 1979). However, drawdown (utilization) of dissolved inorganic carbon (DIC) or nutrients such as dissolved inorganic nitrogen (DIN) for primary production measured as Chl *a* is of greater importance in the application of stable isotopes to infer trophic state. The reason for this is that drawdown represents a temporal integration of volumetric nutrient uptake and utilization and volumetric rate of carbon fixation by phytoplankton (Schelske and Hodell 1991, 1995), whereas standing crop is only an instantaneous measurement at the time and depth of sampling. Organic matter produced and sedimented represents a time-averaged integration of processes affecting stable isotopic composition (Schelske and Hodell 1991). The use of stable isotopes of bulk sediment organic matter to study past environmental conditions is based on the assumptions that sediment organic matter originates from primary production in the water column and that the isotopic ratios reflect those of organic matter produced in the water column (Schelske and Hodell 1991). If these assumptions are valid, then the stable isotopic composition of sediments should be a function of nutrient-driven productivity or trophic state in lakes.

We evaluated relationships between the stable isotopic composition of sediments and water-column variables using sediment samples collected from 83 Florida lakes in 1982 (Brenner and Binford 1988) and data on water-column variables (Huber et al. 1982) from these lakes. Using this set of data it is assumed that measured $\delta^{13}C$ and $\delta^{15}N$ of sediments reflect the isotopic composition of sedimented particulate organic matter produced in the water column. In addition, we determined $\delta^{13}C$ and $\delta^{15}N$ of water and sediment samples from 11 Florida lakes in 1994 to test the assumption that the isotopic composition of sediments reflects the signature of organic matter produced in the water column and the hypothesis that the stable isotope composition of sediments is enriched with increasing lake productivity.

Materials and methods

Surface sediment samples were collected from Florida lakes in 1982 to study relationships between sediment constituents and lake trophic state (see Brenner and Binford 1988). Details of lake selection, sediment sampling, and analyses are presented by Brenner and Binford (1988). Lakes were chosen to reflect a wide range of trophic states and geographic locations within Florida. Surface sediments (top 2 cm), representing the last 2–10 years of deposition, were obtained with an Ekman grab from a mid-lake station in each lake. Organic C, TN, TP, and pigments ($SCDU_{665}$) were determined on the sediment sample from each lake. Median values of water-column variables (Chl *a*, pH, alkalinity, Secchi depth, TN, and TP) for each lake were obtained from data compiled by Huber et al. (1982). One exception was the Chl *a* concentration (59.3 $\mu g \cdot L^{-1}$) of hypereutrophic Lake Apopka. We used the more representative median value (151 $\mu g \cdot L^{-1}$) from a study in 1979–1980 by Canfield (1981). We estimated total DIC and $CO_{2(aq)}$ from data in Huber et al. (1982) with pH–alkalinity and pH–CO_2 relationships (Wetzel and Likens 1991).

Stable isotopes of bulk organic matter in sediments ($\delta^{13}C_{sed}$ and $\delta^{15}N_{sed}$) were determined using a Carlo Erba CNS analyzer interfaced with a PRISM Series II mass spectrometer. For $\delta^{13}C$ analysis, sediment samples from lakes with pH ≥7.5 were acidified with 2 M HCl overnight, rinsed with deionized water, and redried. The remaining samples from lakes with pH <7.5 were not acidified before analysis. Subsamples of ~1 mg were placed in tin capsules and loaded in the Carlo Erba CNS analyzer and combusted at 1040°C in the presence of O_2 in an oxidation column. Combustion gases were passed to a reduction column and the mass spectrometer by a carrier gas. CO_2 was cryogenically concentrated in a triple trap before isotope determination. For $\delta^{15}N$ analysis, subsamples of 5 – 20 mg were combusted and combustion gas was transferred to the mass spectrometer as previously described. Dinitrogen was concentrated in molecular sieves at liquid N_2 temperature.

^{13}C:^{12}C and ^{15}N:^{14}N ratios were determined in the mass spectrometer against internal CO_2 and N_2 standards, respectively. The isotope ratio is expressed in the conventional delta (δ) notation, defined as the per mil (‰) deviation from reference standard PDB limestone or atmospheric N_2. Repeated measurements of a working standard, peptone (Sigma Chemical Co., St. Louis, Mo.), yielded good reproducibility ($\delta^{13}C = -15.8 \pm 0.05$‰ and $\delta^{15}N = 7.0 \pm 0.2$‰) to the reported values (Gu 1993). Acidified and unacidified sediment samples from 14 randomly selected lakes with pH <7.5 showed no significant difference (0.03‰) in $\delta^{13}C$ (paired *t* test, $P = 0.5152$). Duplicate samples analyzed for $\delta^{15}N$ are within instrumental analytical error (0.1‰, $n = 5$).

Water and sediment samples from 11 Florida lakes (hereafter referred to as test lakes) were collected from April to July 1994 to determine the relationship between stable C and N isotopes in planktonic and sediment organic matter and water-column variables. These lakes were selected to represent a broad range in Chl *a* concentrations, i.e., trophic state. Secchi disc depth was measured in the field. Water and surface sediment samples were collected with a Niskin and an Ekman grab sampler, respectively.

Alkalinity and pH were determined by titration and a pH meter, respectively. Total DIC was determined with a model 5011 Carbon Coulometer (UIC Coulometrics, Inc.). Water samples (0.1–0.5 L) used for Chl *a* determinations were filtered using Gelman glass fiber filters that were extracted with 90% acetone for 24 h. The filters were then homogenized and centrifuged. Chl *a* and phaeophytin were determined with a model U–2000 Hitachi spectrophotometer. TP, TN, soluble reactive P (SRP), and DIN (defined as $NH_4^+ + NO_2^- + NO_3^-$) were analyzed using a Technicon II AutoAnalyzer by standard colorimetric methods.

Planktonic organic matter from 0.2 to 2 L of lake water was filtered onto precombusted (600°C) Whatman QM-A quartz filters. Predried (70°C) filters, 1 g of copper oxide, and 1 g of copper wire were loaded into quartz tubes that were evacuated, sealed in a vacuum line, and combusted at 900°C for 1 h in a muffle furnace. Carbon dioxide and N_2 were cryogenically purified in the vacuum line. Particulate organic C (POC) and N (PON) were quantified with a precalibrated MKS manometer connected to the vacuum line. DIC used for isotope determination was extracted from 5 mL of lake water using a modified technique of Kroopnick (1974). Stable C and N isotopes were determined from the purified gases prepared in the vacuum line. Sediment samples were dried and analyzed for stable C and N isotopes as previously described.

Results

Stable isotopes in sediments of 83 lakes

Selected water column and sediment variables show a wide range, reflecting the range in trophic state among our study lakes (Table 1). Chl *a*, a good indicator for primary productivity in Florida lakes (Beaver and Crisman 1991), varied from 1.0 to 152 $\mu g \cdot L^{-1}$. $\delta^{13}C_{sed}$ ranged from –30.6 to –20.4‰, while $\delta^{15}N_{sed}$ ranged from –2.0 to 7.6‰.

We used simple correlation to explore relationships between isotope ratios ($\delta^{13}C_{sed}$ and $\delta^{15}N_{sed}$) and several limnological variables (Table 2). $\delta^{13}C_{sed}$ was significantly

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Table 1. Statistical summary of stable C and N isotope ratios ($\delta^{13}C_{sed}$ and $\delta^{15}N_{sed}$) of sediments and selected limnological variables for 83 Florida lakes.

		$\bar{X}$	SD	n	Max.	Min.	Range
$\delta^{13}C_{sed}$	(‰)	−26.2	2.0	83	−20.4	−30.6	10.2
$\delta^{15}N_{sed}$	(‰)	2.4	2.1	81	7.6	−2.0	9.6
		Water column					
Chl *a*	($\mu g \cdot L^{-1}$)	19.1	30.0	83	152.4	1.0	151.4
TN	($mg \cdot L^{-1}$)	0.82	0.65	82	4.03	0.15	3.88
TP	($mg \cdot L^{-1}$)	0.047	0.064	81	0.420	0.003	0.417
Secchi	(m)	2.00	1.70	80	8.20	0.20	8.00
DIC	($mg \cdot L^{-1}$)	7.06	6.59	81	28.80	0.00	28.8
$CO_{2(aq)}$	($mg \cdot L^{-1}$)	1.66	1.41	76	7.53	0.00	7.53
		Sediment					
Org. matter	(%)	37.1	20.24	80	83.5	1.1	82.4
TC	($mg \cdot g^{-1}$)	196	109	79	476	14	462
TN	($mg \cdot g^{-1}$)	16.4	8.3	70	35.4	2.5	32.9
TP	($mg \cdot g^{-1}$)	1.73	1.65	77	8.09	0.18	7.91
Pigments	($SCDU \cdot g^{-1}$)	0.81	0.10	80	5.90	0.02	5.88

Note: Limnological variables taken from Brenner and Binford (1988), except for DIC and $CO_{2(aq)}$ (see text for calculation methods and abbreviations).

correlated with all water column variables but was not correlated with any of the sediment variables. We used stepwise regression to determine which water-column variables most influence $\delta^{13}C_{sed}$. We first excluded variables that were not correlated with $\delta^{13}C_{sed}$. We then regressed the remaining variables against $\delta^{13}C_{sed}$ and obtained the following model:

$$\delta^{13}C_{sed} = -28.4 + 2.2\ (\pm 0.3)\ \log \text{Chl } a$$

$$(R^2 = 0.375;\ P = 0.0001;\ n = 83)$$

This model shows that Chl *a* explains 38% of the variance of the dependent variable (Fig. 1*a*). Incorporation of additional variables did not significantly reduce the regression residual. $\delta^{15}N_{sed}$ was not significantly correlated with Chl *a* (Fig. 1*b*) or any water-column variable (Table 2) but was significantly correlated with sediment TP (TP_{sed}) and TC (TC_{sed}). Using stepwise regression, we regressed TP_{sed} and TC_{sed} against $\delta^{15}N_{sed}$ and obtained the following equation:

$$\delta^{15}N_{sed} = 2.2 + 2.7\ (\pm 0.3)\ \log TP_{sed}$$

$$(R^2 = 0.24;\ P = 0.0008;\ n = 76)$$

This model explains 24% of the variance of the dependent variable and shows that $\delta^{15}N_{sed}$ is positively related to TP_{sed}.

Stable isotopes of plankton and sediments of the 11 test lakes

Stable isotope ratios of planktonic organic matter ($\delta^{13}C_{plk}$ and $\delta^{15}N_{plk}$) and selected sediment and water-column variables from the 11 test lakes sampled in 1994 varied markedly (Table 3). $\delta^{13}C_{plk}$ ranged from −30.4 to −10.9‰. The most ^{13}C-enriched sample was collected from hypereutrophic Lake Apopka, where our measured Chl *a* was 108 $\mu g \cdot L^{-1}$. The least ^{13}C-enriched sample was obtained from oligotrophic Lake Disston, with a Chl *a* concentration of 2.3 $\mu g \cdot L^{-1}$. $\delta^{15}N_{plk}$ ratios spanned a narrow range from −0.2 to 4.0‰. $\delta^{13}C_{DIC}$ ranged from −13.9 to 12.5‰. The most positive value was derived from Lake Apopka, and the most negative value came from Orange Lake (Chl *a* = 16.5 $\mu g \cdot L^{-1}$).

$\delta^{13}C_{plk}$ was significantly correlated with Chl *a* (Fig. 2*a*) and all other water-column variables except SRP and DIN (Table 4). $\delta^{13}C_{plk}$ and $\delta^{13}C_{sed}$ were also significantly correlated ($r = 0.79$, $P = 0.004$). $\delta^{13}C_{sed}$ was correlated significantly with $\delta^{13}C_{DIC}$ ($r = 0.66$, $P = 0.05$, $n = 9$). Similar to $\delta^{15}N_{sed}$, $\delta^{15}N_{plk}$ was not significantly correlated with Chl *a* or any other variable from the water column. $\delta^{15}N_{plk}$ was only correlated with TN_{sed} but was not correlated with $\delta^{15}N_{sed}$ (Table 4). $\delta^{15}N_{plk}$ increased from oligotrophic to eutrophic lakes but was low in some hypereutrophic lakes (Fig. 2*b*).

For stepwise regression, we eliminated variables that did not correlate significantly with $\delta^{13}C_{plk}$. We also excluded POC because of its strong covariance with Chl *a* ($r = 0.95$, $P = 0.001$). We regressed the remaining variables against $\delta^{13}C_{plk}$ and obtained the following equation:

$$\delta^{13}C_{plk} = -31.0 + 6.4\ (\pm 1.5)\ \log \text{Chl } a$$

$$(R^2 = 0.64,\ P = 0.002,\ n = 11)$$

The model explains 64% of the variance of the dependent variable.

Discussion

Plankton and sediment $\delta^{13}C$

Previous research on relationships between $\delta^{13}C$ and chemical and biological variables indicated that $\delta^{13}C$ of primary producers is related to (i) $\delta^{13}C_{DIC}$, (ii) concentration of $CO_{2(aq)}$, and (iii) photosynthetic C fixation rate (Goericke et al. 1994). In our test lakes, both $\delta^{13}C_{plk}$ and $\delta^{13}C_{sed}$ were correlated significantly with $CO_{2(aq)}$, $\delta^{13}C_{DIC}$, and Chl *a*. Nevertheless, our stepwise regression model revealed that algal biomass (Chl *a*) was the most important factor in the correlation with $\delta^{13}C_{sed}$ in the 83 lakes. A strong correlation between Chl *a* and $\delta^{13}C_{plk}$ from the 11 test lakes further supports data from surficial sediments. Our results are also supported by other studies on relationships between primary productivity and sedimentary $\delta^{13}C$ (Schelske and Hodell 1991; Shemesh et al. 1993) and between growth rate and algal $\delta^{13}C$ (Fry and Wainright 1991; Takahashi et al. 1991). Chl *a*, however, was more strongly correlated with

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Table 2. Correlations between $\delta^{13}C_{sed}$ or $\delta^{15}N_{sed}$ and selected limnological variables from 83 Florida lakes.

	$\delta^{13}C_{sed}$			$\delta^{15}N_{sed}$		
	r	*P*	*n*	*r*	*P*	*n*
Water column						
Chl *a*	0.61	0.001	83	0.07	0.554	81
TN	0.53	0.001	82	0.09	0.447	81
TP	0.49	0.001	81	0.06	0.622	81
Secchi	−0.44	0.003	79	0.11	0.331	79
DIC	0.36	0.012	80	0.02	0.851	80
$CO_{2(aq)}$	−0.32	0.010	75	0.06	0.635	75
Sediments						
Org. C	0.07	0.558	81	0.14	0.233	80
TC	0.15	0.191	77	0.38	0.001	77
TN	0.12	0.592	69	−0.19	0.125	69
TP	−0.02	0.840	76	0.47	0.001	76
Pigments	0.13	0.278	77	0.20	0.239	80

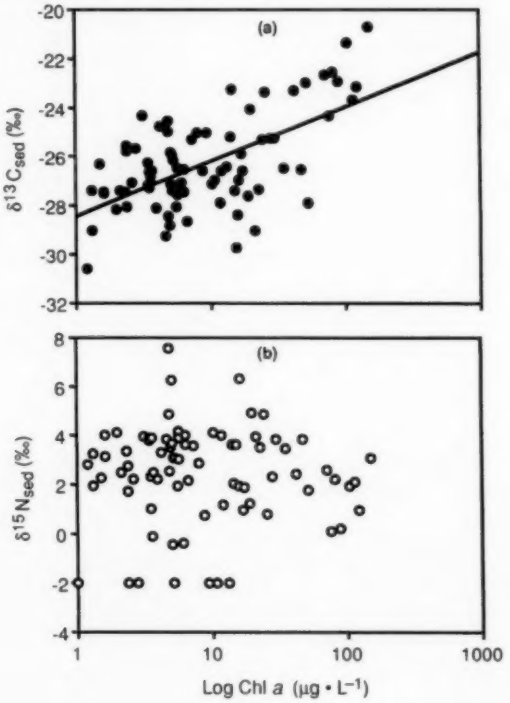
Note: Units and abbreviations as in Table 1.

$\delta^{13}C_{plk}$ ($r = 0.82$) than with $\delta^{13}C_{sed}$ ($r = 0.61$). Indeed, despite a significant correlation between Chl *a* and $\delta^{13}C_{sed}$, 62% of the variance in $\delta^{13}C_{sed}$ of the 83 lakes remains unexplained.

Several factors may explain the strong positive correlation between Chl *a* and $\delta^{13}C_{plk}$ in the test lakes and the poorer relationship between Chl *a* and $\delta^{13}C_{sed}$ in the 83 lakes. Sediment organic matter is not derived solely from plankton. Nonplankton contributions of organic matter to lake sediments may be important. Isotopic ratios in terrestrial carbon generally differ from ratios in planktonic organic matter because photosynthetic pathways (and hence isotopic fractionation) and sources and concentrations of CO_2, as well as forms of inorganic C used in the two ecosystems, are probably different. Studies on sediment $\delta^{13}C$ from estuarine (Shultz and Calder 1976) and coastal regions (Hedges and Parker 1976) indicate various terrestrial C inputs. Analysis of sediment $\delta^{13}C$ from Lake Memphremagog, Quebec, indicated that approximately 40–50% of organic C in the sediment of the open water was terrestrial in origin (LaZerte 1983). Schelske and Hodell (1995) found differences in terrestrial input between two cores collected from the eastern basin of Lake Erie. Small lakes and oligotrophic lakes might be expected to possess relatively high proportions of terrestrial C in their sediments. Sediments of oligotrophic lakes might receive relatively more organic matter of terrestrial origin compared with the contribution from the overlying waters as a consequence of low primary productivity. Sediment $\delta^{13}C$ from a small oligotrophic subalpine lake strongly indicated high terrestrial C input (Rau 1980).

Sediment $\delta^{13}C$ generally reflects the $\delta^{13}C$ of the most abundant primary producers in the environment (Sackett and Thompson 1963). Surface sediment $\delta^{13}C$ was derived from phytoplankton of the overlying water in a temperate estuary (Gearing et al. 1984) and a subarctic marine bay (Goering et al. 1990). However, in macrophyte-dominated lakes, phytoplankton production is typically low, and the major source of organic matter in sediments is aquatic plants that often possess higher $\delta^{13}C$ than do phytoplankton (see review by Fry and Sherr 1984). Isotopic studies in coastal sediments dominated by seagrass indicated that this plant is a significant source of C to the sediments (Fry et al. 1977). Gu et al. (1994) showed that sediment $\delta^{13}C$ in a subarctic lake reflects equal contributions by phytoplankton and macrophytes. A correlation between macrophyte coverage and $\delta^{13}C_{sed}$ was not significant in our lakes ($r = 0.09$, $P = 0.493$, $n = 67$).

Fig. 1. (*a*) Relationship between Chl *a* and $\delta^{13}C_{sed}$. Equation of the least squares regression line is $\delta^{13}C_{sed} = -28.4 + 2.2(\pm 0.3)$ log Chl *a*; $r = 0.61$, $P = 0.0001$, $n = 83$. (*b*) Relationship between Chl *a* and $\delta^{15}N_{sed}$. Correlation is not significant ($r = 0.01$, $P = 0.925$, $n = 81$).



Our results show that $\delta^{13}C_{sed}$ varied little among oligotrophic, mesotrophic, and eutrophic lakes (Scheffé *F* test, $P > 0.05$) despite a wide range of Chl *a* (from <1.0 to 40 $\mu g \cdot L^{-1}$). The mean $\delta^{13}C_{sed}$ between these lakes and the hypereutrophic lakes was significantly different (Scheffé *F* test, all *P*s = 0.001) (Table 5). We propose that $\delta^{13}C_{sed}$ in lakes ranging from oligotrophy to eutrophy is influenced by contributions of nonplanktonic organic matter. In hypereutrophic lakes, however, the input of nonplanktonic organic matter is small compared with in situ phytoplankton production. Quantitative data on terrestrial C inputs to our study lakes are lacking, but the TC:TN ratio of lake sediments indicates that terrestrial inputs affect $\delta^{13}C_{sed}$. This ratio, which is higher in terrestrial plants due to greater structural carbon, is also highest in oligotrophic lakes. An inverse relationship between TC:TN ratio of the lake sediments and water column Chl *a* ($r = -0.45$, $P = 0.001$, $n = 65$) indicates more terrestrial inputs to low productivity lakes. Hypereutrophic lakes selected for this study had <30% macrophyte coverage, a fraction considered to be insignificant with respect to whole-lake primary production (Canfield and Hoyer 1992). These results suggest that nonplankton inputs affect $\delta^{13}C_{sed}$ in Florida lakes, especially in lakes of low productivity.

The broad range of $\delta^{13}C_{DIC}$ in our test lakes (Table 3) may

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Table 3. Statistical summary of $\delta^{13}C$ and $\delta^{15}N$ of planktonic organic matter and selected limnological variables for 11 Florida lakes (test lakes) sampled in 1994.

		$\bar{X}$	SD	n	Max.	Min.	Range
Water column							
$\delta^{13}C_{plk}$	(‰)	−22.7	6.0	11	−10.9	−30.4	19.5
$\delta^{15}N_{plk}$	(‰)	1.8	1.5	10	4.0	−0.2	4.2
$\delta^{13}C_{DIC}$	(‰)	−5.5	8.7	9	12.5	−13.9	26.4
Chl *a*	($\mu g \cdot L^{-1}$)	47.6	44.5	11	113.8	1.0	112.8
POC	($mg \cdot L^{-1}$)	8.9	13.6	11	47.8	0.4	47.4
PON	($mg \cdot L^{-1}$)	0.8	0.8	11	2.7	0.1	2.6
TN	($mg \cdot L^{-1}$)	1.44	1.35	11	4.94	0.19	4.75
DIN	($mg \cdot L^{-1}$)	0.029	0.019	11	0.077	0.003	0.074
TP	($mg \cdot L^{-1}$)	0.106	0.203	11	0.690	0.005	0.685
SRP	($mg \cdot L^{-1}$)	0.010	0.003	9	0.015	0.005	0.010
Secchi	(m)	0.92	0.80	11	2.9	0.20	2.7
DIC	($mg \cdot L^{-1}$)	9.28	9.29	11	26.67	0.42	26.25
$CO_{2(aq)}$	($mg \cdot L^{-1}$)	0.88	1.23	11	0.00	3.81	3.81
Sediment							
TC	($mg \cdot g^{-1}$)	251.9	166.6	11	391.9	5.3	386.6
TN	($mg \cdot g^{-1}$)	23.5	11.3	11	38.9	0.5	38.4
TP	($mg \cdot g^{-1}$)	2.1	2.1	11	7.5	0.1	7.4
$\delta^{13}C_{sed}$	(‰)	−23.8	2.4	11	−20.4	−27.6	7.2
$\delta^{15}N_{sed}$	(‰)	2.7	2.2	11	7.4	0.1	7.3

Note: See text for abbreviations.

indicate varying carbon sources. The highest $\delta^{13}C_{DIC}$ (12.5‰) was measured from Lake Apopka where intensive methanogenesis occurs in the lake sediments (Reddy and Graetz 1991). This extraordinary ^{13}C-rich DIC in turn gives rise to the high $\delta^{13}C_{plk}$ (Fig. 2*a*) and indicates an important supply of DIC from sediment methanogenesis (B. Gu and C.L. Schelske, unpublished data). Conversely, low $\delta^{13}C_{DIC}$ in some lakes may suggest high aerobic respiration or high input of isotopically light terrestrial carbon (Rau 1980). The correlations between Chl *a* and $\delta^{13}C_{sed}$ and $\delta^{13}C_{plk}$ can be confounded by supplies of extremely ^{13}C-enriched or -depleted DIC. Finally, the Chl *a* and $\delta^{13}C_{sed}$ correlation can also be weakened if the Chl *a* data do not represent the trophic state of the lakes. Short-term variability (Carrick et al. 1993) and spatially heterogeneous distribution of Chl *a* (Brenner and Binford 1988) might be responsible for the poor relationship between Chl *a* and $\delta^{13}C_{sed}$.

Several processes may account for the positive correlations between DIC concentration and both $\delta^{13}C_{sed}$ (Fig. 3*a*) and $\delta^{13}C_{plk}$ (Fig. 3*b*). Some algal species can utilize only $CO_{2(aq)}$, which is often a small fraction of DIC in aquatic systems. However, $CO_{2(aq)}$ is inversely related to $\delta^{13}C_{sed}$ and $\delta^{13}C_{plk}$ (Fig. 4). Negative correlation between pCO_2 and $\delta^{13}C_{plk}$ has been observed in marine (Rau et al. 1989) and lacustrine systems (Takahashi et al. 1990). Increases in lake productivity generally deplete $[CO_{2(aq)}]$ and elevate algal $\delta^{13}C$. Nevertheless, it is not clear whether increasing $\delta^{13}C$ at a higher primary productivity level is related to (*i*) decrease in ^{13}C fractionation due to higher C fixation; (*ii*) active CO_2 transport; or (*iii*) HCO_3^- accumulation. Active CO_2 transport as opposed to diffusive CO_2 transport could reduce ^{13}C discrimination during C fixation. The shift from the use of CO_2 to HCO_3^- as a C source may also increase algal $\delta^{13}C$ because HCO_3^- is isotopically heavier than CO_2. Some algal species, especially cyanobacteria, are capable of active CO_2 transport (Miller et al.

Fig. 2. (*a*) Relationship between Chl *a* and $\delta^{13}C_{plk}$. Equation of the least squares regression line is $\delta^{13}C_{plk} = -31.0 + 6.4 \log$ (Chl *a*); $r = 0.82$, $P = 0.002$, $n = 11$. (*b*) Relationship between Chl *a* and $\delta^{15}N_{plk}$. Correlation is not significant ($r = 0.033$; $P = 0.9281$, $n = 10$). Trophic state designations are indicated according to Forsberg and Ryding (1980).

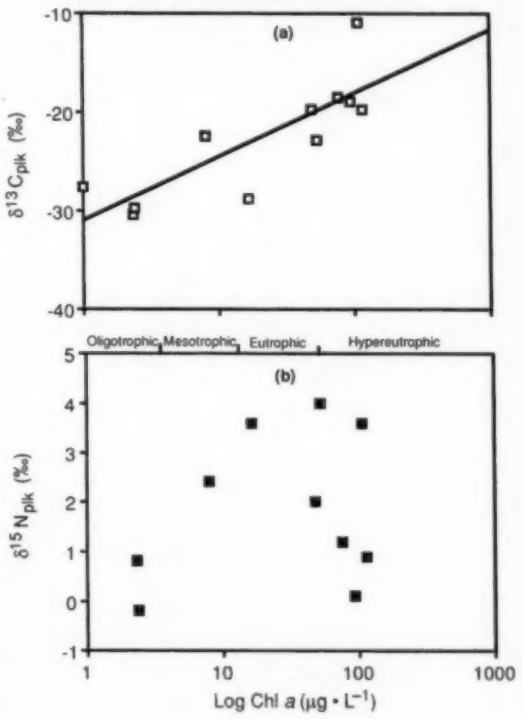

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Table 4. Correlations between $\delta^{13}C_{plk}$ or $\delta^{15}N_{plk}$ and selected limnological variables for the test lakes.

	$\delta^{13}C_{plk}$			$\delta^{15}N_{plk}$		
	r	*P*	*n*	*r*	*P*	*n*
Water column						
Chl *a*	0.82	0.002	11	0.03	0.928	10
POC	0.91	0.001	11	0.22	0.538	10
PON	0.75	0.009	11	0.22	0.538	10
$\delta^{13}C_{DIC}$	0.77	0.016	9	0.41	0.271	9
DIC	0.61	0.045	11	0.35	0.322	10
$CO_{2(aq)}$	–0.60	0.005	11	0.16	0.664	10
TN	0.60	0.040	11	0.27	0.430	10
TP	0.77	0.006	11	0.26	0.463	10
SRP	0.25	0.521	9	0.19	0.651	9
DIN	0.27	0.415	10	0.13	0.725	10
Secchi	–0.77	0.006	11	0.35	0.323	10
Sediments						
$\delta^{13}C_{sed}$	0.79	0.004	11	0.37	0.296	10
$\delta^{15}N_{sed}$	–0.02	0.990	11	0.46	0.184	10
TC	0.48	0.136	11	0.59	0.075	10
TN	0.52	0.102	11	0.65	0.043	10
TP	0.59	0.055	11	0.43	0.214	10

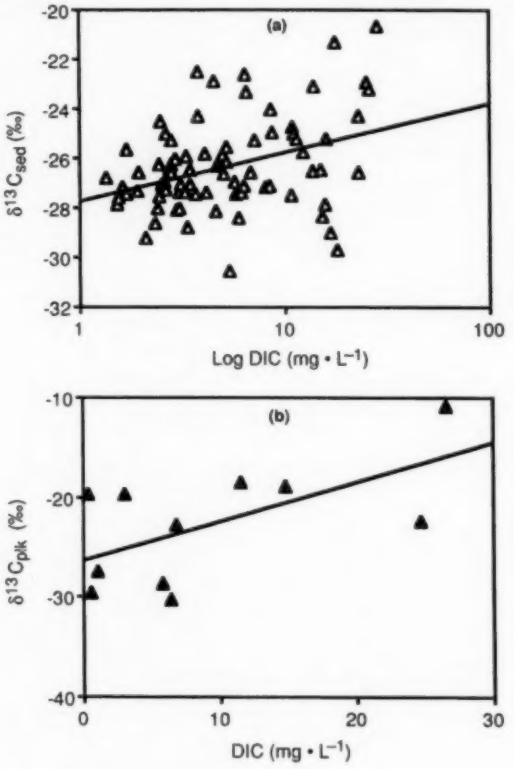
Note: See text for abbreviations and units.

Table 5. Statistical summary of $\delta^{13}C$ and $\delta^{15}N$ (‰) of sediment organic matter from Florida lakes.

	$\delta^{13}C_{sed}$			$\delta^{15}N_{sed}$		
Trophic state	$\bar{X}$	SD	*n*	$\bar{X}$	SD	*n*
Oligotrophic	–27.1	1.5	17	1.4	2.4	17
Mesotrophic	–26.9	1.1	28	2.0	1.9	28
Eutrophic	–26.3	1.6	26	2.4	2.2	25
Hypereutrophic	–23.5	2.0	12	1.9	1.1	11

Note: Data are divided into four groups according to trophic state (Chl *a*) as classified by Forsberg and Ryding (1980).

Fig. 3. (*a*) Relationship between DIC and $\delta^{13}C_{sed}$. Equation of the least squares regression line is $\delta^{13}C_{sed} = -27.7 + 0.2 \log DIC$; $r = 0.405$, $P = 0.0002$, $n = 77$. (*b*) Relationship between DIC and $\delta^{13}C_{plk}$. Equation of the least squares regression line is $\delta^{13}C_{plk} = -26.3 + 0.4$ DIC; $r = 0.612$, $P = 0.045$, $n = 11$.



1991) or utilizing HCO_3^- (Espie et al. 1991). Cyanobacterial biomass increases with trophic state in Florida lakes (Duarte et al. 1992). It is possible that the increase in $\delta^{13}C$ with lake productivity reflects changes in C uptake mechanisms or forms of DIC used. Fogel et al. (1992) suggested that the high $\delta^{13}C$ of phytoplankton from the Delaware Estuary, U.S.A., was a consequence of active CO_2 transport or HCO_3^- use during the high productivity periods when CO_2 was depleted. Zohary et al. (1994) postulated that a dinoflagellate bloom, *Peridinium*, in Lake Kinneret, Israel, might use HCO_3^- via a CO_2-concentrating mechanism. High primary productivity often causes increased DIC demand and decreased CO_2 availability. Changes in $\delta^{13}C_{plk}$ are inevitably linked to changes in primary productivity regardless of whether active CO_2 transport or HCO_3^- accumulation governs the isotopic shift.

Supplies of N or P or both often limit phytoplankton growth. An increase in $\delta^{13}C$ may be expected when greater N or P availability stimulates algal C fixation. Decreases in ^{13}C fractionation by terrestrial and aquatic plants with increases in inorganic N supply have been documented (Bender and Berge 1979; Raven and Farquhar 1990). Fry and Wainright (1991) showed that addition of silica significantly stimulated marine diatom growth and increased $\delta^{13}C$. Significant correlations between TN and TP of the overlying water and $\delta^{13}C_{sed}$ (Fig. 5) suggest that primary productivity in Florida lakes increases with TN or TP or both. Schelske and Hodell (1991) found that changes in sediment $\delta^{13}C$ in Lake Ontario were driven by increased P loading and P abatement at different times. Relationships between TN and TP and Chl *a* in Florida lakes (Beaver and Crisman 1991; Canfield 1983; Kratzer and Brezonik 1981; Shannon and Brezonik 1972) also support our finding that increase in $\delta^{13}C_{sed}$ with trophic state is dependent on nutrient (N or P) supply.

Plankton and sediment $\delta^{15}N$

It has been reported that $\delta^{15}N$ of phytoplankton is a function of substrate level and growth rate (Cifuentes et al. 1989; Wada and Hattori 1978). However, our data show no significant relationship between Chl *a*, TN, or DIN and $\delta^{15}N_{sed}$ and $\delta^{15}N_{plk}$. $\delta^{15}N_{sed}$ might be affected by nonplankton contributions of organic N (Sweeney and Kaplan 1980; Owens and Law 1989). Nevertheless, this cannot explain the lack of correlation between selected water-column variables and $\delta^{15}N_{sed}$. Instead, other processes may be important determinants of $\delta^{15}N_{sed}$ variability in aquatic systems.

A number of factors have been reported to affect $\delta^{15}N$ of

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Fig. 4. (*a*) Relationship between $CO_{2(aq)}$ and $\delta^{13}C_{sed}$. Equation of the least squares regression line is $\delta^{13}C_{sed} = -26.2 - 1.5 \log CO_{2(aq)}$; $r = 0.36$, $P = 0.009$, $n = 75$. (*b*) Relationship between $CO_{2(aq)}$ and $\delta^{13}C_{plk}$. Equation of the least squares regression line is $\delta^{13}C_{plk} = -20.1 - 2.9\ CO_{2(aq)}$; $r = -0.596$, $P = 0.053$, $n = 11$.

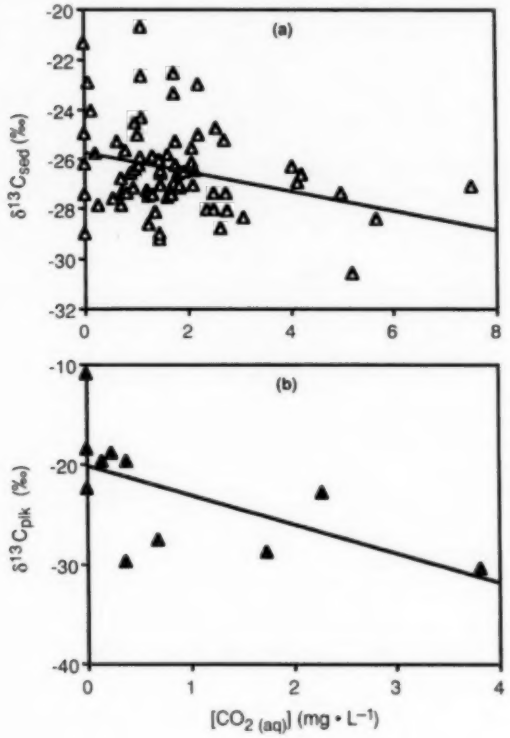
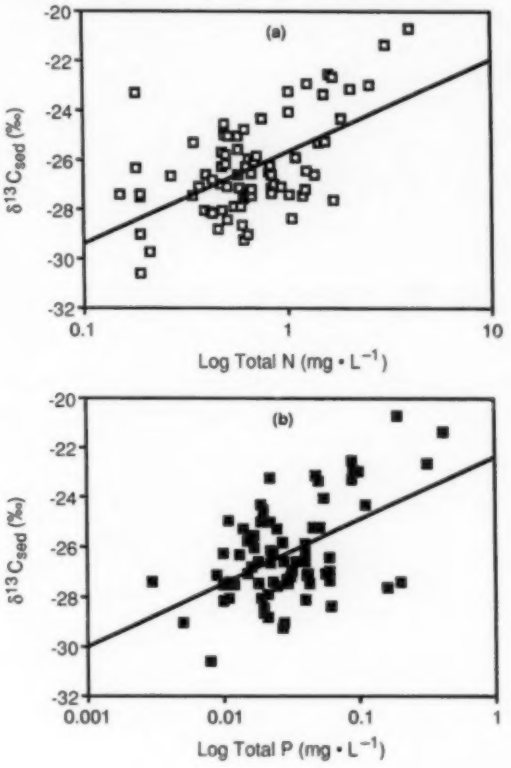


Fig. 5. (*a*) Relationship between TN of the water column and $\delta^{13}C_{sed}$. Equation of the least squares regression line is $\delta^{13}C_{sed} = -25.7 + 3.4 \log TN$; $r = 0.53$, $P = 0.0001$, $n = 82$. (*b*) Relationship between TP of the water column and $\delta^{13}C_{sed}$. Equation of the least squares regression line is $\delta^{13}C_{sed} = -22.6 + 2.4\ TP$; $r = 0.49$, $P = 0.001$, $n = 81$.



planktonic organic matter. $\delta^{15}N$ of phytoplankton was inversely related to NH_4^+ concentration in a subarctic lake (Gu 1993). A negative relationship between NO_3^- concentration and $\delta^{15}N$ of sediment (Altabet and Francois 1994) or plankton (Altabet and McCarthy 1985; Goering et al. 1990) has been documented. Algal N demand can also affect ^{15}N fractionation. A linear relationship between algal growth rate and $\delta^{15}N$ was found in laboratory studies of marine diatoms (Wada and Hattori 1978). Field studies also show significant relationships between primary productivity and $\delta^{15}N$ of plankton and sediment in lacustrine (Gu 1993) and marine environments (Calvert et al. 1992; Shemesh et al. 1993). Nevertheless, lakes of various productivity levels are dominated by different groups of photosynthetic organisms that are capable of utilizing various N pools and may fractionate ^{15}N differently. The N_2-fixing cyanobacteria show little isotope fractionation and have $\delta^{15}N$ similar to that of atmospheric N_2, i.e., 0‰ (Peterson and Fry 1987). Algae that do not fix N_2 typically exhibit greater variation in $\delta^{15}N$, depending on the isotopic ratio of the N source, size of N pools, and the extent of isotope fractionation (Gocricke et al. 1994; Owens 1987). The algal community in Florida lakes tends to shift from green algal dominance in oligotrophic lakes, to diatom dominance in mesotrophic lakes, and to cyanobacterial dominance in eutrophic and hypereutrophic lakes (Duarte et al. 1992). Thus the $\delta^{15}N$ of the algal community may change in response to the shift in dominant groups.

Other studies also indicate that seasonal variability of $\delta^{15}N_{plk}$ can be explained in part by changes in phytoplankton composition. Lower $\delta^{15}N$ is associated with N_2-fixing cyanobacterial species, such as *Anabaena* (Gu et al. 1994) and *Trichodesmium* (Minagawa and Wada 1986). However, in lakes dominated by non-N_2 fixing algae, $\delta^{15}N$ is largely determined by lake productivity. Our results show increases in $\delta^{15}N$ from oligotrophic to eutrophic lakes and a decrease in hypereutrophic lakes (Table 5 and Fig. 2*b*). This pattern is more evident in $\delta^{15}N_{plk}$ of the test lakes than in $\delta^{15}N_{sed}$ of the 83 lakes because sediments are potentially altered by nonplankton organic matter inputs as discussed previously.

The $\delta^{15}N$ signature of planktonic organic matter may be affected by the degree of N limitation because N-fixing cyanobacteria are likely to be present in N-limited lakes with adequate supplies of other nutrients. The difference in $\delta^{15}N_{plk}$ between eutrophic lakes and oligotrophic lakes is larger than that between eutrophic lakes and hypereutrophic lakes

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(Table 5), suggesting that primary productivity is a stronger determinant of $\delta^{15}N$ than changes in species composition as in our test lakes. This is probably true because eutrophic and hypereutrophic lakes are not always dominated by N_2-fixing cyanobacteria. Furthermore, the rate of N_2 fixation is controlled by several environmental factors, including N loading, water column stability, and temperature. For instance, the $\delta^{15}N$ (3.6‰) of hypereutrophic Lake Apopka was relatively high, reflecting the low rate of N_2 fixation reported by Reddy and Graetz (1991). This might be attributed to the frequent, wind-induced water-column mixing in this shallow N-limited lake (Aldridge et al. 1993; Carrick et al. 1993) that may inhibit N_2 fixation. Nevertheless, the nonlinearity of $\delta^{15}N$ with lake trophic state suggests an overall pattern of N limitation and N_2 fixation in some eutrophic and hypereutrophic Florida lakes.

Conclusions

Our study shows that several limnological variables (DIC, $CO_{2(aq)}$, $\delta^{13}C_{DIC}$, TP, and TN) are significantly correlated with $\delta^{13}C$ of water column and sediment organic matter. Regression analysis suggests that algal biomass (i.e., Chl *a*) is the most important factor determining the variability in both $\delta^{13}C$ of plankton and sediments. Increases in lake primary productivity, as reflected by algal standing crop, are correlated with higher $\delta^{13}C$ of phytoplankton. Nutrients (C, N, and P) are important factors affecting lake productivity and $\delta^{13}C$ of phytoplankton. Nonplanktonic inputs of organic matter from either terrestrial or macrophytic plants may reduce the strength of $\delta^{13}C_{sed}$ as an indicator for lake productivity. $\delta^{13}C_{DIC}$ provides important information on metabolic process (i.e., aerobic versus anaerobic respiration) and carbon sources in lakes. Our results suggest that the increase in $\delta^{15}N$ of planktonic and sediment organic matter with trophic state is confounded by the presence of N_2-fixing cyanobacteria in productive lakes. $\delta^{15}N$ is controlled by primary productivity of non-N_2-fixing algae in lakes with adequate supplies of N and by N_2 fixation of N_2-fixing cyanobacteria in more eutrophic lakes that are N-limited. Thus low $\delta^{15}N$ is not determined simply by primary productivity but may occur in both low and extremely high productivity environments. Interpretation of $\delta^{15}N$ data, therefore, depends on information on algal community structure and nutrient (e.g., N and P) supplies. Our conclusion that the nonlinearity of $\delta^{15}N$ across a large trophic gradient of freshwater lakes is dependent on the importance of water-column N_2 fixation needs to be investigated with additional research.

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Ontogenic habitat shifts of the Iceland scallop, *Chlamys islandica* (Müller, 1776), in the northern Gulf of St. Lawrence

David J. Arsenault and John H. Himmelman

Abstract: The spatial distribution of the Iceland scallop, *Chlamys islandica* (Müller, 1776), was studied using scuba in an unharvested population in the Mingan Islands, northern Gulf of St. Lawrence. Size partitioning related to depth was evident, there being a change from predominantly small scallops (<30 mm in shell height) at 15 m to mainly large scallops (>60 mm) at 30 m in depth. Most small scallops were found in crevices under bivalve shells or rocks. Size partitioning did not appear to result from differential mortality among depths. Tethering trials suggested that size-specific predation pressure was similar between experimental depths (15 and 25 m). Vulnerability to predators and the frequency of spatial refuge use both decreased markedly with increasing scallop size and this suggested an ontogenetic microhabitat shift related to predation risk. The survival of small scallops in shallow waters (15 m) may be enhanced because of the abundance of bivalve shells that could provide refuges from predators. The depth distribution of rhodophytes, a settlement substratum, largely accounted for the distribution of recruits. We propose that size partitioning in this population results from higher settlement and survival in shallow water, followed by a gradual downslope movement with increasing scallop size.

Résumé : Nous avons étudié la distribution spatiale du pétoncle d'Islande, *Chlamys islandica* (Müller, 1776), en plongée autonome (scuba), dans une population non-exploitée de l'archipel de Mingan, dans le nord du golfe du St-Laurent. La répartition bathymétrique variait selon la taille : les pétoncles <30 mm dominaient en faible profondeur (15 m) et les individus de >60 mm dominaient plus en profondeur (30 m). La majorité des petits pétoncles se trouvaient dans des crevasses sous des coquilles de bivalves et des roches. La répartition bathymétrique selon la taille ne semblait pas découler de mortalité différentielle entre les profondeurs. Des expériences de prédation in situ ont suggéré que la pression de prédation sur différentes tailles de pétoncles était similaire entre les profondeurs expérimentales (15 et 25 m). La vulnérabilité à la prédation ainsi que l'utilisation de refuges spatiaux diminuait avec l'augmentation en taille des pétoncles, suggérant un changement ontogénique de microhabitat ajusté au risque de prédation. La distribution de rhodophytes, un substrat pour la fixation larvaire, expliquait en grande partie la distribution bathymétrique des recrues. La survie des recrues pourraient être favorisée en faible profondeur (15 m) par l'abondance de coquilles de bivalves, si ces dernières procurent un refuge contre les prédateurs. Nous proposons que la répartition spatiale des différentes tailles de pétoncles dans cette population est le résultat d'une fixation larvaire accrue et d'une survie supérieure des recrues en faible profondeur, suivit d'un mouvement graduel vers les profondeurs avec l'augmentation en taille des pétoncles.

Introduction

Spatial partitioning of juvenile and adult stages, which results from selection of specific breeding grounds by the adults, is frequently encountered in mobile marine invertebrates (Woodbury 1986; Hines et al. 1987; Himmelman and Dutil 1991; Wahle and Steneck 1991). For marine invertebrates with planktonic larval development, the distribution of spawning adults may not determine the distribution of recruits because hydrodryamic processes may cause gametes and larvae to drift over considerable distances (on the scale of kilometres) (Sinclair 1988). Following their development in the plankton, competent larvae of benthic invertebrates move near the bottom and settle on suitable substrata (Thorson 1950). Consequently, the distribution of suitable settlement substrata potentially influences the distribution of spat and juveniles.

Predation on post-settlement stages may further affect distributional patterns by restricting juveniles to specific habitats (Keough and Downes 1982; Kotler 1984; Connell 1985; Richards 1992) or by favouring the development of antipredator behaviours (Sih 1987). In habitats with high predation pressure, where the probability of surviving an encounter with a predator is low, the evolution of avoidance behaviours (such as the use of spatial refuges) is favoured because it enhances survival (Sih 1987). When the vulnerability to predators changes with size, avoidance behaviours are often adjusted according to predation risk (Sih 1982; Richardson and Brown 1992; Wahle 1992). This suggests that prey can evaluate predation risk and adjust their antipredator behaviours adaptively (Lima and Dill 1990).

The Iceland scallop, *Chlamys islandica* (Müller, 1776), is characteristic of cold-water regions around Norway, Iceland, Greenland, and eastern Canada (Ekman 1953). It is generally found attached by byssal threads to coarse substrata in areas with strong tidal currents (Wiborg 1963; Vahl and Clausen 1980). In contrast to scallops such as *Placopecten magellanicus*

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D.J. Arsenault[1] and J.H. Himmelman. Groupe interuniversitaire de recherches océanographiques du Québec (GIROQ) and Département de biologie, Université Laval, QC G1K 7P4, Canada.

[1] Author to whom all correspondence should be addressed.

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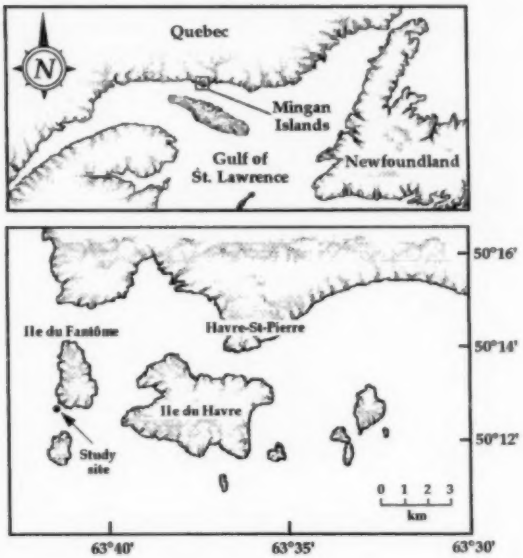
Fig. 1. Location of the population of Iceland scallops, *Chlamys islandica*, at Île du Fantôme in the Mingan Islands, northern Gulf of St. Lawrence, eastern Canada.



and *Argopecten irradians*, byssal attachment is maintained through the adult stage, presumably to avoid being swept away by currents (Vahl and Clausen 1980). Nevertheless, *Chlamys islandica* can free itself from byssal attachment to move over short distances (Vahl and Clausen 1980) or to avoid predators (D.J. Arsenault, personal observations).

Over the past 20 years, the commercial exploitation of *Chlamys islandica* has expanded greatly in eastern Canadian waters (Giguère and Miller 1993), but little is known about its biology. Recent studies have focused on its reproductive cycle (Giguère et al. 1994), larval settlement (Harvey et al. 1993), and the association of adults with different substrata (Gilkinson and Gagnon 1991). Studies on its population dynamics are only reported for Norwegian fjord populations (Wiborg 1963; Gruffydd 1976*a*, 1976*b*; Vahl 1982). For Canadian waters, the only population data available are from fisheries stock assessments made using scallop dredges (reviewed by Giguère and Miller 1993).

The present study used scuba diving to investigate factors influencing the size structure and spatial distribution of *Chlamys islandica*, in the Mingan Islands, northern Gulf of St. Lawrence, eastern Canada. Specifically we examined (*i*) size-related depth distribution, (*ii*) distribution of natural settlement substrata, (*iii*) ontogenetic changes in substratum utilization, (*iv*) distribution of potential scallop predators, and (*v*) vulnerability to predation related to size and depth.

Materials and methods

We studied population characteristics of *Chlamys islandica* at the southern side of Île du Fantôme in the Mingan Islands (50°13′6″N, 63°41′12″W; Fig. 1). During the summers of 1992 and 1993, we quantified the spatial distribution of scallops along a permanent transect, placed perpendicular to the shore, extending from 5 to 30 m in depth. In both years, we sampled 20 quadrats of 0.25 m^2 that were randomly placed within a 30-m wide horizontal strip at each of six depths along the transect (5, 10, 15, 20, 25, and 30 m in depth). Because scallops were often cryptic, we carefully examined the undersides of all loose materials (gravel, rocks, and shells) within each quadrat. For each scallop encountered we noted whether it was hidden or exposed and its association with the substratum (95% were byssally attached to a given substratum) and then placed it in a numbered plastic bag for subsequent determination of shell height. The substratum categories used were sand (0–5 mm), gravel (5–25 mm), small rocks (25–100 mm), large rocks (>100 mm), and shells. We further visually estimated the percentage cover of each of these substrata in each quadrat. In 1992, to evaluate variations in mortality with depth, we also collected the valves of dead scallops present in each quadrat. We sorted the latter in the laboratory and only recent mortalities, i.e., shells with a noneroded and nonfouled nacreous layer, were considered for analysis.

Scallops were grouped into five size-classes (0–15, 15–30, 30–45, 45–60, and >60 mm in shell height). The distribution with depth of the different scallop sizes were compared using a χ^2 test. For each size-class collected in 1992, we calculated the correlation (r_s, Spearman's rank correlation) between the depth distribution of dead and living scallops. Negative correlations would suggest that the distribution with depth of living scallops results from differential mortality among depths. Size-related variations in the proportion of cryptic scallops were analyzed using a χ^2 test. For each scallop size, χ^2 goodness-of-fit tests were used to test whether overall substratum use was proportional to availability (calculated as the relative surface area of all quadrats sampled). Although sand was the most abundant substratum at the study site, covering 36% of the bottom, no scallops were found this substratum. Thus, sand was probably not a potential substratum for byssal attachment and was therefore excluded as a category in this analysis (analyses in which sand was included gave similar conclusions). Departures from proportional utilization would indicate that scallops selected or avoided certain substrata. Finally, we compared substratum utilization among scallop size-classes using a χ^2 test.

Spawning at Île du Fantôme occurs abruptly (within 7–10 d) in late July (unpublished data for 1991 to 1993), and settlement is likely to occur in the autumn. (We observed newly metamorphosed spat on artificial collectors in mid-October in 1992; unpublished data.) In early June 1993, we determined the depth distribution of hydroids and red algae (Rhodophyta), which are potential natural settlement substrata for *Chlamys islandica* (Harvey et al. 1993). Algal diversity at the study site was low and the rhodophyte *Ptilota serrata* dominated (>95% of the algae). Other less abundant algae were the rhodophytes *Phyllophora truncata* and *Phycodrys rubens*. For analytical purposes, all rhodophytes were considered as one group. At each depth, we collected hydroids and red algae in 20 quadrats (0.25 m^2). In the laboratory, we counted the spat (from the previous autumn's settlement) and determined the dry mass (after 6 h at 50°C) of the hydroids and rhodophytes. We did not directly assess the association of spat with these particular substrata because the substrata were often intertwined and further because the spat occasionally became detached during transport to the laboratory. Shell height of spat was measured using a calibrated dissecting microscope. In analyzing settlement preference, biomass may be misleading because settlement intensity may be related to surface area (Harvey et al. 1993). For this reason, we made no direct comparisons between substrata. If the spatial distribution of spat is determined by the distribution of a given substratum, then spat density should vary with substratum availability, irrespective of its surface area. Thus, we used the degree of correlation between spat density and substratum biomass as an index of the substratum's importance in determining spat distribution. We calculated the partial correlation coefficients between scallop spat density, settlement substrata biomass, and depth ($n = 120$). Partial correlation, a measure of correlation between pairs of variables when other

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variables are held constant, determines the specific association between each pair of variables, excluding potential interactive effects with the other variables (Zar 1984). The amount of variability in spat distribution accounted for by depth, rhodophyte biomass, and hydroid biomass was calculated using coefficients of determination (the square of the partial correlation coefficients, Zar 1984). Variations with depth in mean biomass of settlement substrata (g/0.25 m^2), in spat density (spat/0.25 m^2), and in spat shell height were examined using Kruskal–Wallis tests, followed by Dunn's multiple comparisons tests ($\alpha = 0.05$; Zar 1984). The association between mean density of spat (collected on hydroids and rhodophytes) and of small scallops (<15 mm in shell height, collected on the bottom in 1993) was determined using Pearson's correlation coefficient ($n = 6$).

In 1992 and 1993, we evaluated the density of potential predators of scallops by sampling 1-m^2 quadrats at each of the six depths. The number of quadrats sampled in 1992 was 61, 70, 200, 69, 105, and 45 at 5, 10, 15, 20, 25, and 30 m in depth, respectively, and 40 quadrats per depth in 1993. Potential predators included the asteroids *Leptasterias polaris*, *Crossaster papposus*, and *Asterias vulgaris* and the crabs *Hyas araneus* and *Cancer irroratus*. The lobster *Homarus americanus*, a known predator of scallops (Elner and Jamieson 1979) was absent in the study site. Sampling of crabs was limited to individuals measuring >2 cm in maximum carapace dimension because smaller individuals were often hard to observe because of heavy colonization on the carapace by epibionts.

To evaluate the effects of depth on size-specific predation pressure, tethering experiments (Heck and Wilson 1987) were carried out in June and July 1993. Scallops were tethered by attaching a 50-cm long monofilament thread to a hole that was drilled in the auricle. The size groups tested measured 15–30, 30–45, 45–60, 60–75, 75–90, and 90–105 mm in shell height. At both 15 and 25 m in depth, 20 scallops of each size group were tethered at random positions at 1-m intervals to transects that were placed parallel to the shore. Natural spatial refuges were highly scattered so that all tethered scallop would not have had the same chance to finding them. Thus, to decrease variation in the estimates of mortality, we removed all potential refuges in the vicinity of tethered scallops. To evaluate experimental mortality (bias), we examined the survival of 20 tethered scallops of different sizes maintained in a cage. Tethering has the advantage of allowing identification of individual scallops, and causes of mortality can be assessed by the characteristics of the remains. After a 21-d period, we examined the state of the tethered scallops (dead or living) and classified the valves of dead scallops as crushed or intact. We assumed that crushed valves resulted from decapod predation (Lake and Jones 1987; Barbeau and Scheibling 1994*a*) and intact valves from asteroid predation or other sources of mortality (Stokesbury and Himmelman 1995). Because tethering can influence the escape response of scallops to various predator species differently (Barbeau and Scheibling 1994*b*), comparisons of mortality sources may be biased by this experimental procedure. However, if predators attacking scallops are the same in different treatments, tethering provides a good measure of relative vulnerability (Barbeau and Scheibling 1994*b*). We compared the relative vulnerability of different-sized scallops between depths using separate log-linear models (multidimensional χ^2 analysis) for decapod predation (crushed valves) and asteroid predation (intact valves).

Results

At Île du Fantôme, *Chlamys islandica* was virtually absent at 5 and 10 m in depth but abundant between 15 and 30 m, where size partitioning related to depth was evident (Figs. 2, 3). Differences in the relative abundance of the different sizes of scallops were detected between 1992 and 1993 (Fig. 2) and were likely due to interannual differences in recruitment intensity or to sampling variability. Nevertheless, a general pattern was evident (Fig. 3). The distribution of <30 mm scallops was strongly skewed toward shallower depths (15 m) and with increasing size, the distribution shifted towards greater depths (Fig. 3).

The substratum in the study area varied markedly with depth (Fig. 4). At 5 m, the bottom was sand-covered bedrock with scattered gravel and cobbles. At 10 m, sand predominated and sand dollars, *Echinarachnius parma*, and endobenthic bivalves (mainly *Spisula polynyma* and *Mya truncata*) were abundant. At 15 m, the bottom was heterogeneous with scattered gravel, rocks, and shells of *Spisula polynyma* on sand. The shells and gravel were often cemented together by the tube-forming polychaete *Amphitrite johnstoni*. From 20 to 30 m in depth, the bottom was relatively homogenous, with *Lithothamniun*-encrusted rocks and gravel dominating.

The proportion of cryptic scallops decreased markedly with increasing scallop size ($\chi^2_{(4)} = 254.3$, $P < 0.0001$), from 85% for 0–15 mm scallops to 0% for >60 mm scallops (Fig. 5). Most cryptic scallops (80%) were on the undersides of bivalve shells, mainly shells of *Spisula polynyma*.

Substratum use was different ($P \leq 0.0002$) from availability except for 45–60 mm scallops ($P = 0.139$, Fig. 6). The lack of a substratum preference for the latter group was probably due to their low numbers ($n = 25$). Further, the distribution of scallops on the different substrata varied with size (Fig. 6; $\chi^2_{(12)} = 155.4$, $P < 0.0001$). Smaller scallops (<30 mm) were mostly attached to shells and larger scallops (>30 mm) to small rocks and gravel (Fig. 6). Associations with gravel and small rocks increased with increasing scallop size. Most scallops associated with small rocks and gravel (100 and 87%, respectively) were on the surface of the substratum. For all sizes of scallops, the association with large rocks was low (9–13%) and similar to availability (13%). Whereas shells covered only 4% of the bottom, over 75% of 0–15 mm scallops were associated with shells. Most (96%) were byssally attached to the undersides of the shells. With increasing size, the association with shells decreased markedly and was only 5% for >60 mm scallops.

The density of spat found on settlement substrata was correlated with the biomass of these substrata and not with depth (Table 1). The abundance of rhodophytes and hydroids accounted for 52 and 23%, respectively, of the variations in spat density, whereas depth only accounted for 0.1% of the variation. The density of spat varied markedly with depth ($H = 26.3$, $P < 0.0001$), being maximal (≈3.5 spat/0.25 m^2) at 15 m and much less (0–0.9 spat/0.25 m^2) at other depths (Fig. 7). The biomass of settlement substrata also varied with depth ($H = 67.2$, $P < 0.0001$, for rhodophytes and $H = 64.1$, $P < 0.0001$, for hydroids), and the depth distribution of rhodophytes was most similar to the distribution of scallop spat (Fig. 7), being markedly more abundant at 15 m (5.2 g/0.25 m^2) than at all other depths (0–1.2 g/0.25 m^2). Hydroids were nearly absent at 5 and 10 m in depth, and their abundance did not vary significantly between 15 and 30 m (Fig. 7). The distribution of small scallops (<15 mm in shell height) was positively correlated with the distribution of scallop spat ($r = 0.88$, $P = 0.02$, $n = 6$). Spat shell height did not vary with depth ($H = 2.84$, $P = 0.41$), and overall mean shell height was 3.15 mm (SE = 0.24).

The depth distribution and relative abundance of the different potential predators was similar between 1992 and 1993 (Fig. 8). The asteroid *Asterias vulgaris* and the decapod *Cancer*

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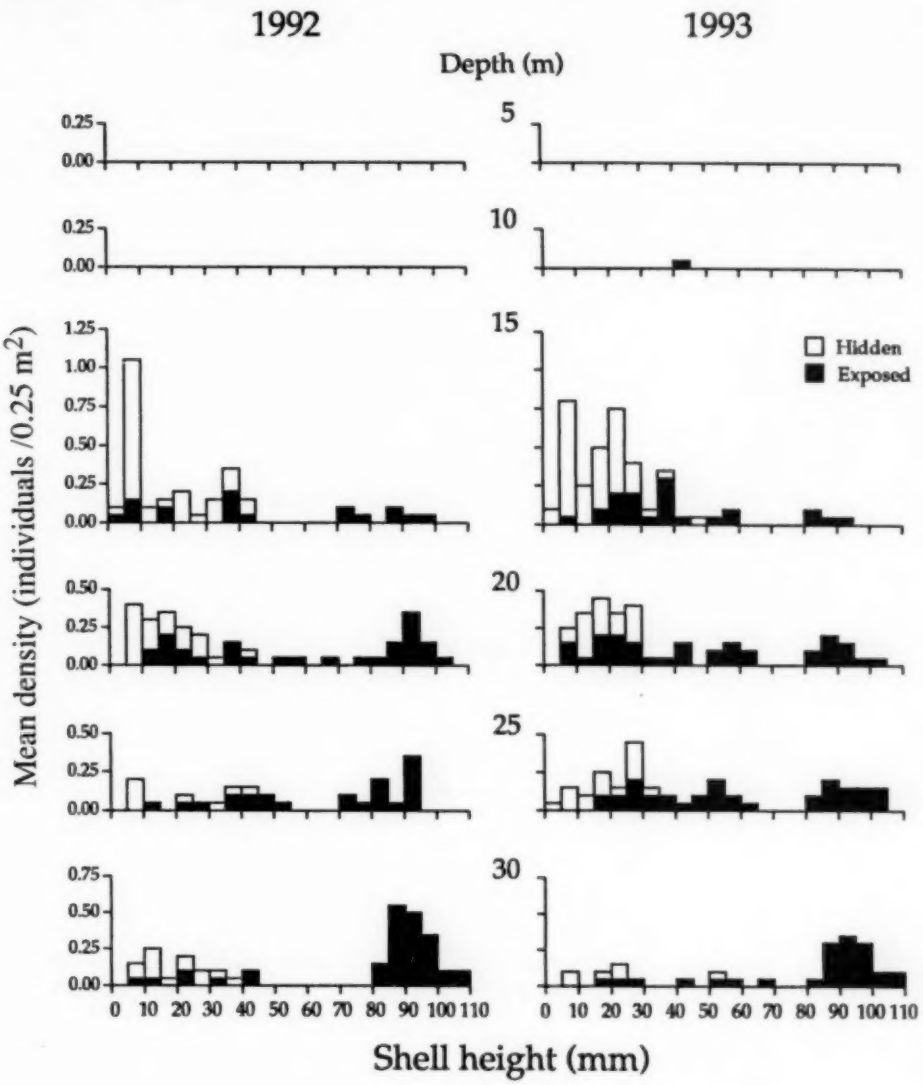
Fig. 2. Size structure relative to depth of Iceland scallops, *Chlamys islandica*, at Île du Fantôme, Mingan Islands, northern Gulf of St. Lawrence, determined by sampling 20 quadrats of 0.25 m^2 at each depth in summer 1992 and 1993.



irroratus were uncommon and limited to shallow waters, whereas other predators were present at all depths. The most abundant predators were the asteroids *Leptasterias polaris* and *Crossaster papposus* and the decapod *Hyas araneus* (Fig. 8). In both years, the density of both small (<15 cm) and large (>15 cm) *Leptasterias polaris* was maximal at 5 m in depth. At depths where scallops were found (>10 m), small *Leptasterias polaris* did not show any consistent depth distribution between years, whereas the density of larger individuals was relatively uniform with depth and between years. The density of small *Crossaster papposus* was generally low (<0.1 individual/m^2), and their depth distribution showed a similar pattern between years, with maximal abundance at 20 m in depth. In both years, no consistent pattern of depth distribution was found for larger *Crossaster papposus*. Finally, *Hyas araneus* was generally more abundant at depths >10 m, where densities varied from 0 to 0.15 individuals/m^2.

The analysis of the tethering data indicated that size-specific predation pressure was similar between depth. The interaction between scallop size and depth was nonsignificant for both decapod ($P = 0.197$) and asteroid ($P = 0.179$) predators (Table 2). The analysis also indicated that decapod predation may be higher at 15 than at 25 m ($P = 0.067$). Vulnerability to predators decreased with increasing scallop size (Fig. 9). This decrease was more pronounced for decapod predation (crushed valves) as the analysis indicated a marked size effect

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Fig. 3. Depth distribution of *Chlamys islandica* of different sizes at Île du Fantôme, Mingan Islands, determined by sampling 20 quadrats of 0.25 m^2 at each depth in summer 1992 and 1993. χ^2 values are for comparisons of the depth distribution among the different scallop size-classes.

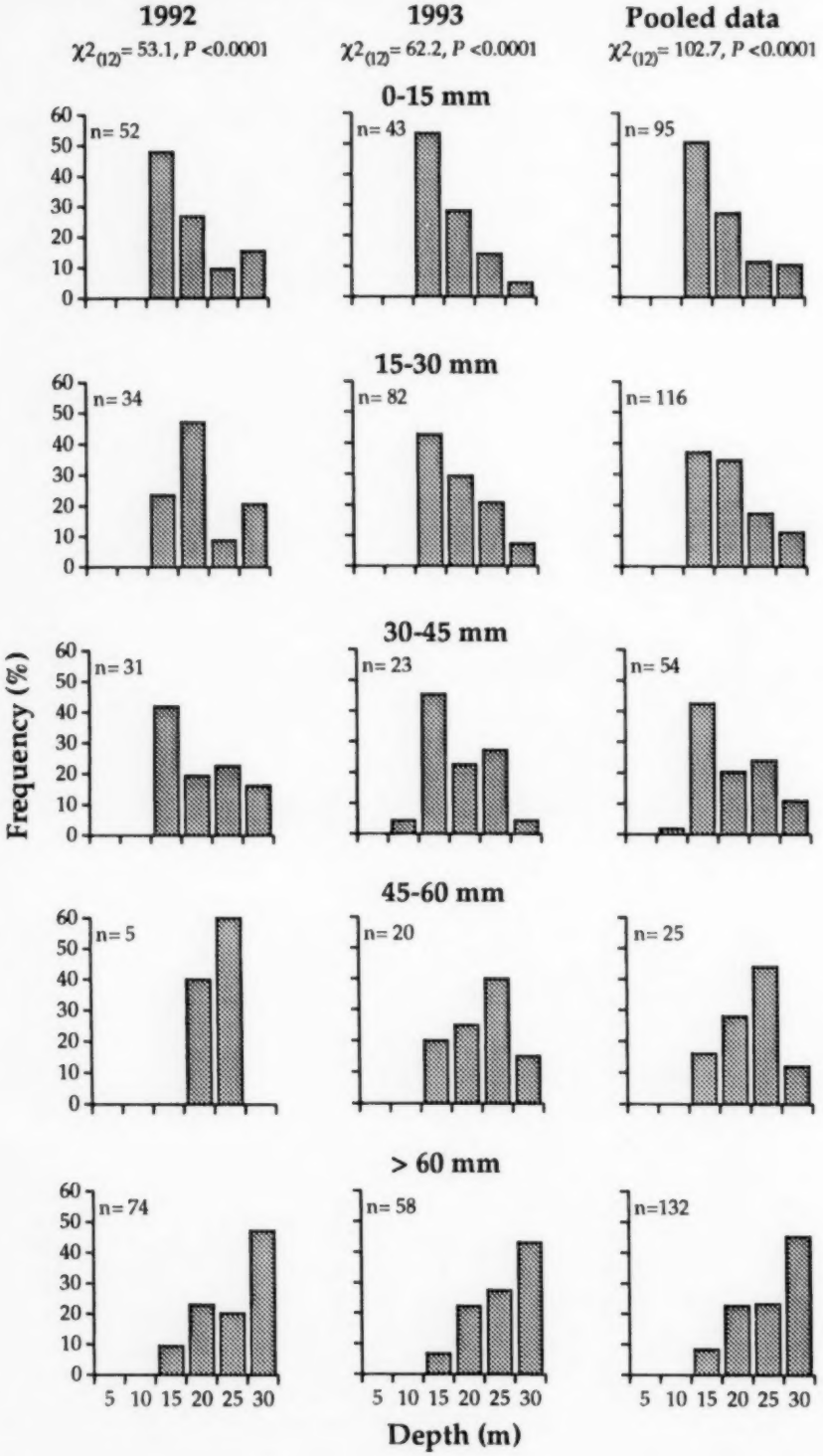

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Fig. 4. Mean cover area of different substrata in relation to depth at Île du Fantôme, Mingan Islands, where the population of Iceland scallops, *Chlamys islandica,* is located. Values are from pooled data for 1992 and 1993 and vertical bars represent standard errors (n = 40 for each depth).

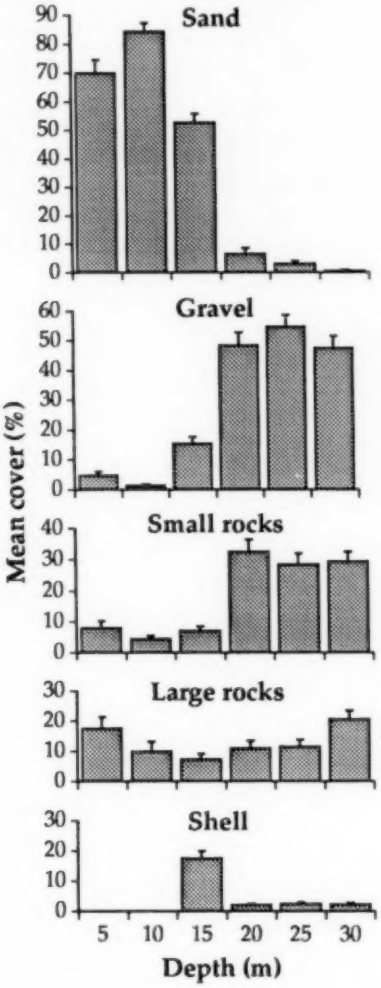
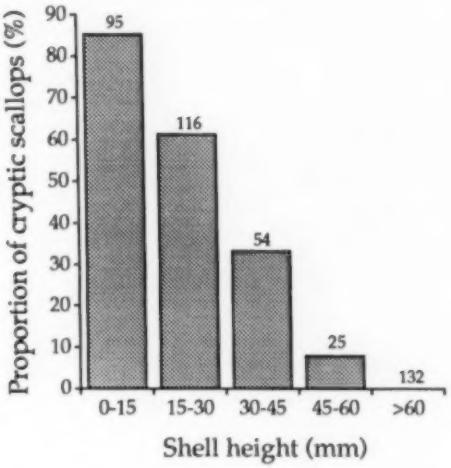


Fig. 5. Size-related variations in the proportion of cryptic Iceland scallops, *Chlamys islandica,* at Île du Fantôme, Mingan Islands. Values were calculated from pooled data collected in 1992 and 1993 by sampling 20 quadrats of 0.25 m^2 at six depths in both years.



for crusted valves (P <0.0001) and a slight and nonsignificant effect for intact valves (P = 0.058; Fig. 9). Intact valves can result from either asteroid predation or other sources of mortality. Since no mortalities were observed for tethered scallops maintained in a predator exclusion cage, we assumed that intact valves resulted from asteroid predation. Most <30 mm scallops that were preyed upon had crushed valves, whereas most larger scallops that were preyed upon had intact valves (Fig. 9). For all size-classes sampled in 1992, the distributions of dead and living scallops with depth were positively correlated (r_s = 0.53–0.96; Table 3).

Discussion

A scarcity of juveniles in scallop beds has been reported for many species (Brand 1991). This may be attributed to the selectivity of sampling methods, especially when sampling is performed using commercial dredges (Baird and Gibson 1956). Sampling using less selective dredges, lined with small mesh netting, or scuba diving can improve the evaluation of population structure (Thouzeau et al. 1991*a*, 1991*b*; Kenchington et al. 1991). Spatial partitioning has been reported to occur in some scallop species (Wiborg 1963; Gruffydd 1976*b*; Thouzeau and Lehay 1988; Kenchington et al. 1991). For populations with strong spatial size partitioning, sampling at specific depths, as is frequent in fisheries surveys, is likely to generate misleading population structure data (D'Amours and Pilote 1982; Giguère et al. 1990; Giguère and Miller 1993). Our sampling using scuba showed a marked size partitioning with depth for Iceland scallops at Île du Fantôme. The size structure varied from a predominance of small scallops at the upper edge of the population (15 m) to mainly large scallops at 30 m in depth.

Size partitioning of *Chlamys islandica* at Île du Fantôme did not appear to result from differential mortality between depths. The distribution of potential predators was relatively uniform throughout the zone where scallops were found (15 to 30 m), and our tethering trials suggested that size-specific predation pressure was similar with 15 and 25 m in depth. If the local abundance of predators regulates the distribution of scallops, the low occurrence of predators at 10 m in depth should have favoured the establishment and survival of scallops at this depth. However, no scallops were found there. The similar distribution of dead and living scallops with depth further suggests that the observed size partitioning is not explained by differential predation.

Scallop larvae are known to settle on a wide range of substrata (Larsen and Lee 1978; Brand et al. 1980; Minchin 1992). The abundance of settlement substrata may strongly influence the distribution of early benthic stages (Wahle and

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Fig. 6. Size-related variations in the frequency of association with different substrata by Iceland scallops, *Chlamys islandica*, at Île du Fantôme, Mingan Islands. (A) Availability of the different hard substrata and (B) size-specific associations with the various substrata. For each scallop size, χ^2 values are for the comparison of observed and expected (proportional to availability) frequency of associations with the different substrata.

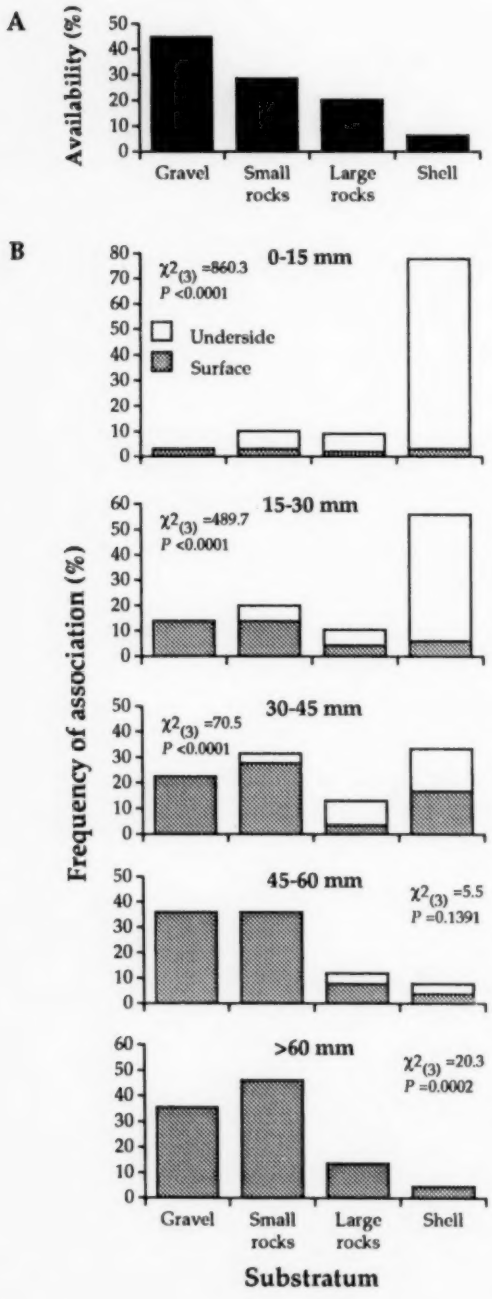

Table 1. Partial correlation coefficients (n = 120) between the density of spat of Iceland scallops, *Chlamys islandica*, collected on settlement substrata, the biomass of settlement substrata (rhodophytes and hydroids), and depth calculated from 20 quadrats of 0.25 m^2 sampled at 5, 10, 15, 20, 25, and 30 m in depth during summer 1993 at Île du Fantôme, Mingan Islands.

	Scallop spat density	Rhodophyte biomass	Hydroid biomass	Depth
Scallop spat density	—	—	—	—
Rhodophyte biomass	0.72**	—	—	—
Hydroid biomass	0.48**	−0.10	—	—
Depth	0.04	−0.16	0.30*	—

*P = 0.001.
**P < 0.0001.

Fig. 7. Depth distribution of settlement substrata (rhodophytes and hydroids) and Iceland scallop spat, *Chlamys islandica*, at Île du Fantôme, Mingan Islands. Values with the same letters form homogenous groups as determined by Dunn's multiple comparisons tests following significant Kruskal–Wallis tests.

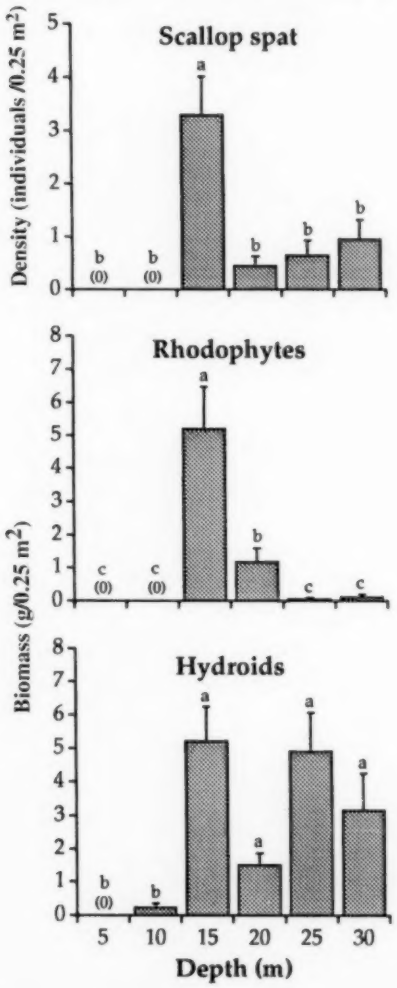

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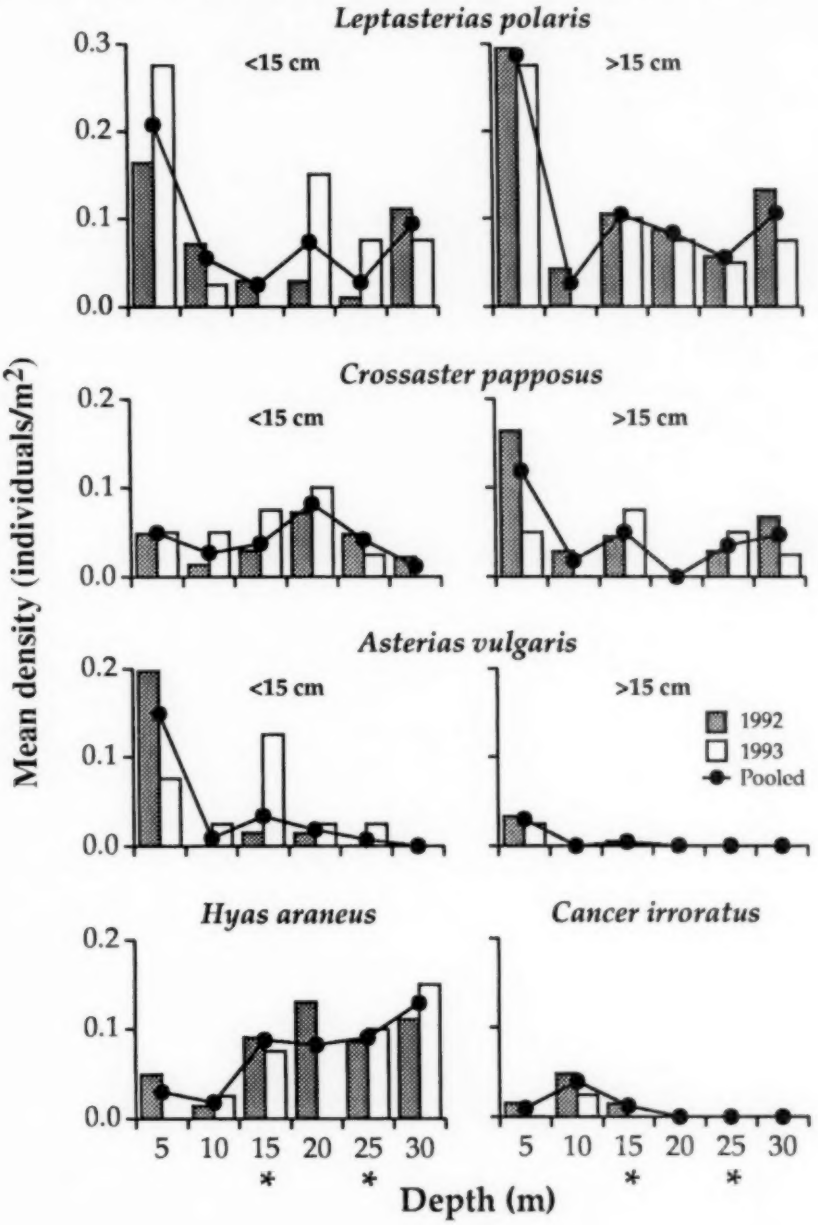
Fig. 8. Mean density of potential predators of the Iceland scallops, *Chlamys islandica*, at Île du Fantôme, Mingan Islands, in 1992, 1993, and for the 2 years together. Asterisks below the horizontal axis indicate the depths at which the tethering experiments were performed in 1993.



Steneck 1991; Minchin 1992). At Île du Fantôme, the distribution of rhodophytes accounted for 52% of the variation in spat density. Whereas Harvey et al. (1993), from studies in the southern Gulf of St. Lawrence, reported that Iceland scallop larvae preferentially settle on the hydroid *Tubularia larynx* (particularly on dead perisarcs), our data show that the distribution of hydroids is less likely to explain the distribution of spat; it only accounted for 23% of variation in spat density. In our study the predominant hydroid was *Sertularia* sp., and *Tubularia larynx* was absent. Our data suggest that the distribution of recruits is determined by the distribution of settlement substrata. We observed a high degree of association between the depth distribution of spat and that of small scallops (<15 mm). The scarcity of small scallops at <15 m in

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Table 2. Summary of log-linear models relating the frequency of mortality, as determined from tethering studies, to size and depth for Iceland scallops, *Chlamys islandica*, at Île du Fantôme, Mingan Islands, during summer 1993.

Source of mortality	Source of variation	df	Likelihood χ^2	P
Decapods	Size	5	32.48	<0.0001
	Depth	1	3.35	0.067
	Size × depth	5	7.34	0.197
Asteroids	Size	5	10.70	0.058
	Depth	1	0.60	0.437
	Size × depth	5	7.61	0.179

depth is likely a result of the low abundance of settlement substrata there (likely because of the mainly sandy bottom).

On attaining a certain size, scallop spat detach from their settlement substratum and drop to the bottom (Minchin 1992; Garcìa-Esquivel and Bricelj 1993). These young scallops are likely to be vulnerable to predators until they reach a refuge in size (Dayton 1971; Werner et al. 1983). The utilization of habitats that minimize predation risk should be favoured by natural selection because it increases the probability of surviving to reproductive size. We observed that small scallops (<30 mm) were strongly aggregated under rocks and bivalve shells (Fig. 6) that probably reduce predation risk (Barshaw and Lavalli 1988; Dumbauld et al. 1993; Fernandez et al. 1993; Skilleter 1994). Predator avoidance behaviours are thought to have evolved when the probability of surviving an encounter with a predator is low (Sih 1987). If using spatial refuges reduces the vulnerability of Iceland scallops to predators, survival of small individuals would likely be greater at 15 m depth at Île du Fantôme because of the high abundance of shell refuges there.

With increasing prey size, vulnerability to predators and the number of potential predators often decrease because a refuge in size is attained (Werner and Gilliam 1984). This appears to be true for Iceland scallops. Our tethering experiments indicate that the predators attacking scallops change as the scallops increase in size. Predation by decapods is frequent for small scallops, but scallops become largely invulnerable to decapods at about 30 mm. Juanes (1992) suggests that the decrease in attacks by decapods with increasing prey size results from mechanical limitations of the chela. The size selectivity we observed for decapod predation was not likely to be biased by the tethering procedure because the response of scallops to encounters with decapods is generally passive and limited to valve closure (Barbeau and Scheibling 1994*b*; D.J. Arsenault, personal observations). This is further indicated because a similar pattern of size selectivity was observed in cage experiments in which untethered Iceland scallops of different sizes were exposed to predation by the crabs *Hyas araneus* and *Cancer irroratus* (unpublished data). In contrast with decapod predation, predation by asteroids in our tethering experiments was generally low and only decreased slightly with increasing size (Fig. 9). As with other scallop species, *Chlamys islandica* shows a strong escape response when it encounters asteroid predators (Legault and Himmelman 1993). Barbeau and Scheibling (1994*b*) show that tethered scallops may be limited in their ability to escape asteroid predators. If the degree to which the escape response is limited

Fig. 9. Size-specific mortality of Iceland scallops, *Chlamys islandica*, determined by tethering experiments at Île du Fantôme, Mingan Islands. Presented values are pooled data for 15 and 25 m in depth (Table 3, $n = 40$ per size-class). Values sharing the same letters are not significantly different according to Tukey-type multiple comparisons test (Zar 1984).

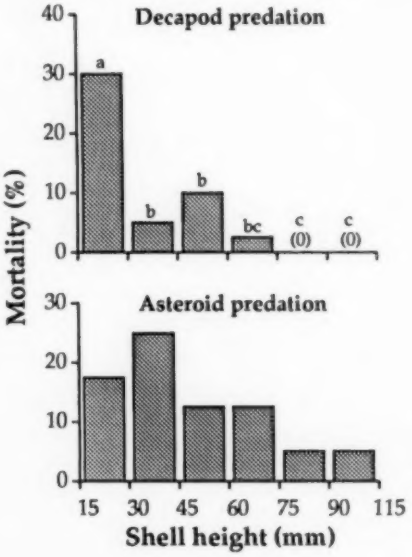

Table 3. Comparison of the distribution with depth of different sized living (L) and dead (D) Iceland scallops, *Chlamys islandica*, at Île du Fantôme, Mingan Islands, sampled in 20 quadrats of 0.25 m^2 at each depth in summer 1992.

Depth	Shell height (mm) 0–15		15–30		30–45		45–60		>60	
(m)	L	D	L	D	L	D	L	D	L	D
5	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0
15	25	6	8	6	13	6	0	3	7	6
20	14	2	16	13	6	12	3	4	17	2
25	5	0	3	0	7	4	2	2	15	2
30	8	0	7	2	5	7	0	2	35	9
r_s	0.86		0.96		0.53		0.61		0.81	
P	0.055		0.033		0.237		0.173		0.072	

Note: Spearman's correlation coefficient (r_s) and its associated probability (P) are presented for each scallop size-class.

varies with scallop size, the size-related vulnerability to asteroids we document may be biased.

Habitat shifts during ontogeny often are associated with size-related variations in predation risk and with variations in feeding opportunities in different habitats (Werner and Gilliam 1984). Conflicting demands (minimizing predation risk and maximizing growth opportunities) often force prey species to make trade-offs between survival and growth when selecting habitats. Size-related variations in the frequency of spatial refuge use by *Chlamys islandica* are correlated with their vulnerability to predators outside of these refuges (Figs. 5, 9). This suggests that *Chlamys islandica* undergoes

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an ontogenetic shift in microhabitat use related to predation risk. Spatial refuge use by the scallop *Argopecten irradians* is reported to involve a trade-off between survival and growth (Ambrose and Irlandi 1992). Small individuals attach to blades of seagrass to avoid predators (Pohle et al. 1991), but their elevated position places them in conditions less favorable for growth (Ambrose and Irlandi 1992). Vulnerability to predators decreases with increasing size (Tettelbach 1986). *Argopecten irradians* progressively attaches to lower positions on the blades and eventually becomes free-living on the bottom (Tettelbach 1986; Pohle et al. 1991; Garcìa-Exquivel and Bricelj 1993). We are presently investigating whether the use of spatial refuges by Iceland scallops involves a trade-off between survival and growth.

Most bivalves are sessile, but swimming has evolved in Pectinidae and other families as modifications of mantle cleansing mechanisms (Yonge 1936). Swimming is produced by rapid opening and closing of the valves, which forces a jet of water out of the mantle cavity (Moore and Trueman 1971). Many workers believe that swimming has evolved to permit rapid escape from predators once there has been an encounter (Ansell 1969; Yonge and Thompson 1976). Such escape responses are particularly well developed in scallops (Brand 1991). However, swimming may also be an adaptation to permit movement to more favourable habitats (Winter and Hamilton 1985). Movement over long distances (100 m to km) has been suggested to occur for many scallop species (reviewed by Brand 1991). These locomotory capacities could influence their distribution and spatial partitioning (Fairbridge 1953; Wiborg 1963; Gruffydd 1976*b*). Some researchers suggest that young scallops may develop in nursery areas and later move to adult beds (Mason 1957; Dadswell and Weihs 1990). The ability of scallops to swim changes with size and the optimum is generally attained during the juvenile stage (Gruffydd 1976*b*; Dadswell and Wihs 1990). Gruffydd (1976*b*) showed that 30-40 mm *Chlamys islandica* swim more frequently than larger and smaller individuals and that increases in current enhance swimming activity for individuals of all sizes. Marked spatial partitioning has been reported for several Norwegian populations of *Chlamys islandica*, where larger scallops are found in the inner parts of fjords and smaller individuals in shallower waters at the entrance (Wiborg 1963). Gruffydd (1976*a*, 1976*b*) suggests that a downstream drift of *Chlamys islandica* larvae causes recruitment at the entrance and is followed by directional movement of juveniles to the inner fjord. The latter may be enhanced by strong upstream compensation currents during spring and summer. Our data suggest that recruitment of Iceland scallops at Île du Fantôme is maximal at 15 m in depth. This is also where shell debris is abundant, which likely enhances survival of recruits. With increasing scallop size, the depth distribution shifts to greater depths (Fig. 3). Thus, we hypothesize that the spatial partitioning of Iceland scallops at Île du Fantôme results from higher settlement and survival in shallow waters followed by a gradual downslope movement with increasing size.

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Longitudinal changes of macroinvertebrate communities along an Appalachian stream continuum

J.W. Grubaugh, J.B. Wallace, and E.S. Houston

Abstract: Richness and mean annual abundance and biomass of benthic taxa were measured in prevalent habitats along a first- through seventh-order stream continuum in the southern Appalachian Mountains (United States). Richness was greatest in midorder reaches, and benthic densities were highest in cobble habitats, followed by bedrock, pebble–gravel, and depositional areas. Abundance-based estimates of functional feeding-group composition were dominated by collector–gatherers and changed little with habitat. In contrast, biomass-based estimates differed greatly among habitats: collector–filterers generally dominated cobble and bedrock areas; collector–gatherers, in pebble–gravel; and shredders and collector–gatherers, in depositional habitats. When functional-group biomass estimates were weighted for relative habitat availability along the continuum, patterns of benthic community composition generally matched predictions of the river continuum concept (RCC), although localized changes in stream geomorphology also influenced community structure. Habitat-weighted abundance estimates did not produce similar trends. Because RCC predictions are based on benthic biomass, caution and qualification must be exercised when using abundance data to test RCC predictions.

Résumé : On a déterminé la richesse ainsi que l'abondance et la biomasse annuelle moyenne des taxons benthiques dans des habitats prévalants le long d'un continuum fluviatile d'ordre une à sept dans le secteur sud des Appalaches (É.-U.). La richesse était maximale dans les passages d'ordre moyen, tandis que les habitats à cailloux, suivis des aires constituées du substratum rocheux, de galets–gravier, et les aires sédimentaires présentaient la densité benthique la plus élevée. Des valeurs estimatives, basées sur l'abondance, de la composition des groupes d'alimentation fonctionnels indiquaient qu'il y avait surtout des organismes collecteurs et que la composition ne changeait guère d'un habitat à l'autre. Par contraste, les valeurs estimatives basées sur la biomasse variaient considérablement d'un habitat à l'autre; les collecteurs–filtreurs dominaient généralement dans les aires recouvertes de cailloux et celles constituées du substratum rocheux, tandis que ce sont les collecteurs et les déchiqueteurs et collecteurs qui dominaient dans les aires de galets–gravier et les habitats sédimentaires, respectivement. Lorsque les valeurs estimatives de la biomasse des groupes fonctionnels étaient pondérées pour tenir compte de la disponibilité relative des habitats le long du continuum, la composition de la communauté benthique correspondait généralement aux prévisions du concept du continuum fluviatile (RCC), même si des changements localisés dans la géomorphologie du cours d'eau influaient également sur la structure de la communauté. Les valeurs estimatives pondérées pour tenir compte de l'habitat n'ont pas indiqué des tendances semblables. Comme les prévisions du concept du continuum fluviatile (RCC) sont fondées sur la biomasse benthique, il faut faire preuve de prudence et nuancer les conclusions lorsqu'on utilise des données d'abondance pour vérifier les prévisions RCC.
[Traduit par la Rédaction]

Introduction

Stream ecosystems provide an array of changing physical conditions that influence benthic fauna. Some characteristics such as elevation, thermal regime, and stream size change predictably along undisturbed stream gradients and sequentially alter benthic community structure (Vannote et al. 1980; Minshall et al. 1985). Geomorphic characteristics such as streambed slope, substratum composition, and current velocity often change more locally and with less predictability (Statzner and Higler 1986). These characteristics determine the relative availability of stream habitats such as bedrock outcrops, cobble riffles, pebble–gravel runs, and depositional areas (Brussock et al. 1985). Benthic communities are adapted to utilize the structural and resource features of particular habitats (Huryn and Wallace 1987*a*), and geomorphic characteristics thereby influence benthic community structure on a local or stream-segment scale. The interplay of longitudinal and localized physical characteristics produce distinct patterns in the structure of benthic communities along stream continua.

Changes in benthic community structure can be assessed by examining taxonomic composition along stream continua. Allan (1975*a*, 1975*b*) characterized species composition along an elevational stream gradient in Colorado, U.S.A., and used an index of community similarity to identify specific stream

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J.W. Grubaugh,[1] J.B. Wallace, and E.S. Houston.[2]
Department of Entomology and Institute of Ecology, The University of Georgia, Athens, GA 30602, U.S.A.

[1] Author to whom all correspondence should be addressed: Department of Biology, Division of Ecology and Organismal Biology, The University of Memphis, Memphis, TN 38152, U.S.A.
[2] Present address: Alabama Department of Environmental Management, Ecological Studies Field Operations, 1890 Dickinson Drive, Montgomery, AL 36107, U.S.A.

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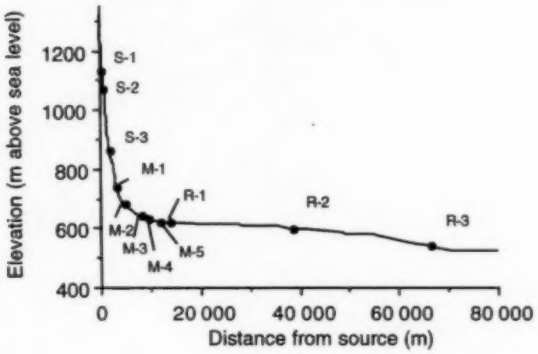
Fig. 1. Longitudinal profile of the Ball Creek – Coweeta Creek – Little Tennessee River continuum. Locations of sampling stations in small streams (S-1 through S-3), midorder (M-1 through M-5), and river (R-1 through R-3) reaches are denoted along the gradient.



reaches exhibiting rapid faunal replacement. Statzner and Higler (1986) applied a similar approach to published lists of benthic taxa from a worldwide assortment of elevational gradient studies. They also identified abrupt changes in species composition and attributed resulting zonation patterns to localized changes in "stream hydraulics," or the physical characteristics of streamflow (Statzner and Higler 1986).

Longitudinal patterns of community structure are also indicated by changes in the functional feeding-group composition of the benthos. These functional feeding groups refer primarily to modes of feeding (Cummins 1973; Wallace et al. 1992) or the food acquisition system (sensu Cummins 1986). This approach was used in formulating the river continuum concept (RCC), a series of hypotheses that suggest a general, predictable pattern of changing benthic community structure in stream size continua (Vannote et al. 1980; Minshall et al. 1985). Accordingly, subsequent studies that support, dispute, or rely on RCC predictions as an appropriate description of lotic ecosystems also are based on functional feeding-group composition. Minshall et al. (1983) examined functional composition of benthic communities along four North American stream-size gradients and found that longitudinal patterns agreed with those predicted by the RCC. Community composition along an extensive continuum in the Salmon River system (572 km, second- to eighth-order reaches) also supported the basic premises of the RCC (Minshall et el. 1992). Conversely, Ward (1986) found little conformance of functional feeding-group composition in St. Vrain River to the RCC. Townsend and Hildrew (1984) found a predictable arrangement of functional composition in small-stream continua in England but hypothesized it was in response to food resource and predation factors rather than evolutionary processes suggested by the RCC. In the Rhine River, van der Velde and van den Brink (1994) compared longitudinal composition of benthic functional structure with RCC predictions to assess whether this anthropogenically impacted river retained the characteristics of a river ecosystem.

There are limitations in some studies that use functional composition to assess benthic community structure. Benthic sampling has often been confined to a single habitat, such as cobble riffles, at all sampling stations along a stream continuum. This approach provides information on how taxonomic or functional structure within a single habitat changes along the gradient but does not account for benthic populations in unsampled habitats (Minshall et al. 1985). As habitat availability changes both locally and longitudinally, it is doubtful that samples from a single habitat can adequately reflect community composition throughout a stream continuum. An alternative approach is to assess relative habitat availability at each sampling station, focus sampling efforts in the dominant habitats at each station, and proportionally weight results by relative habitat availability to reflect the total benthic community (sensu Huryn and Wallace 1987*a*).

Predictions of the RCC concerning functional composition of communities along stream-size continua were based on measures of macroinvertebrate biomass (see Vannote et al. 1980, p. 132). Despite this, many studies that test RCC predictions use measures of benthic abundances rather than biomass (see also Towers et al. 1994). Estimates of functional composition based on abundance and biomass data can be very different (Lugthart and Wallace 1992); therefore, using benthic abundance data to test predictions of the RCC is inappropriate and even possibly misleading.

The objective of this study is to characterize the structure of the benthic community along a continuous elevational and stream-size gradient in the southern Appalachian Mountains. Our approach examines three aspects of the benthic community structure. First, we assess taxonomic composition and patterns of faunal replacement with respect to changes in stream elevation along the continuum. Second, using measures of macroinvertebrate biomass, we describe longitudinal trends in functional-group composition for specific habitats along the stream-size gradient. Finally, we characterize total (habitat-weighted) functional composition of benthic communities along the continuum, using estimates of both macroinvertebrate biomass and abundance. We will compare these results with predicted longitudinal patterns of benthic community structure suggested by the hypotheses of the RCC.

Study area

The study was conducted in Macon and Swain counties of western North Carolina in the Blue Ridge province of the southern Appalachian Mountains. Eleven sampling stations were established along a continuous, 65-km stream continuum that included Ball Creek from its headwaters, the entire length of Coweeta Creek, and a reach of the Little Tennessee River between the Coweeta Creek confluence and Fontana Reservoir (Fig. 1). The Ball Creek –Coweeta Creek – Little Tennessee River continuum flows northwest over granitic parent materials and ionic concentrations in streamwater are persistently low (see Swank and Waide 1988; Swank and Bolstad 1994). Sampling stations can be partitioned into three reaches: three stations (S-1 through S-3) in the small-stream reach, five stations (M-1 through M-5) in the midorder reach, and three stations (R-1 through R-3) in the river reach (Fig. 1).

Stations in the small-stream reach include first- (headwater) and second-order sites along Ball Creek (Table 1). The stream is narrow (<2 m), shallow (<0.25 m), and steeply sloped, with bryophyte-covered bedrock outcrops, cobble-riffle sections, and depositional areas formed behind channel obstructions such as woody debris. The canopy over Ball Creek consists of

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Table 1. Physical parameters at sampling stations along the Ball Creek – Coweeta Creek – Little Tennessee River continuum.

Station code[e]	Stream system	Stream order	Catchment area (ha)	Elevation (m above sea level)	Water-surface slope (%)	Mean annual discharge ($m^3 \cdot s^{-1}$)	Mean width (m)[a]	Mean depth (cm)[a]	Annual degree-days	Prevalent habitat types
S–1	Ball Creek	1	12	1 356	38.6	0.01	0.5	4	3454[c]	Bedrock, cobble, depositional
S–2	Ball Creek	2	39	1 159	21.2	0.02	3.8	8	3568[c]	Bedrock, cobble, depositional
S–3	Ball Creek	2	119	847	9.4	0.10	5.1	12	3794[c]	Bedrock, cobble, depositional
M–1	Ball Creek	4	690	690	7.0	0.27	5.5	18	3937[c]	Bedrock, cobble, depositional
M–2	Coweeta Creek	5	1 548	671	2.9	0.58	7.2	25	4078[c]	Bedrock, cobble, depositional
M–3	Coweeta Creek	5	3 052	642	0.43	1.06[b]	13	25	4322[c]	Cobble, depositional
M–4	Coweeta Creek	5	4 163	633	0.53	1.35[b]	15	25	4389[d]	Cobble, depositional
M–5	Coweeta Creek	5	4 456	622	0.43	1.43[b]	13	30	4401[d]	Pebble–gravel, depositional
R–1	Little Tennessee River	6	36 260	620	0.05	10.85	25	50	4763[c]	Cobble, depositional
R–2	Little Tennessee River	7	83 660	597	0.14	22.18[b]	60	50	4922[d]	Bedrock, cobble, depositional
R–3	Little Tennessee River	7	112 900	540	0.35	29.54	70	50	5064[c]	Bedrock, depositional

[a]Bankfull measurements.
[b]Estimated mean discharge from linear regression equation ($y = 0.26 + 2.62 \times 10^{-4}$ (catchment area), $n = 6$, $r^2 = 0.998$).
[c]Based on temperatures from 2-h interval thermistor recordings for a 2-year period (J.B. Wallace, unpublished data).
[d]Estimated from linear regression equation using temperature data from other stations ($y = 2909 + \log_{10}$(catchment area), $n = 8$, $r^2 = 0.969$).
[e]Station codes are given in Fig. 1.

mixed hardwoods and rosebay rhododendron (*Rhododendron maximum*) that effectively shades the stream year round. The small-stream reach is of relatively short length (<5 km) but includes an elevational change of over 500 m (Fig. 1). Water temperatures, determined from recording thermistors deployed along the continuum (J.B. Wallace, unpublished data), vary little and annual degree-days are less than 3800 for all small-stream stations. Catchment areas above each station range from 10 to 100 ha (Table 1). More complete descriptions of the Ball Creek watershed are given by Huryn (1986) and Houston (1994).

Midorder reach stations were established along the fourth-order section of Ball Creek and the 7-km length of fifth-order Coweeta Creek (Table 1). Streambed slope is moderate as this reach encompasses the transitional zone between erosional mountain streams and alluvial river valley (Fig. 1). Average stream depth is ~0.5 m; width varies between 5 and 15 m with very little channel braiding. Canopy cover progressively diminishes downstream and annual degree-days range between ~3900 and 4400. Bryophyte-covered bedrock outcrops persist at upstream stations (M-1 and M-2) but not further downstream. Cobble riffles and depositional areas are common at stations M-3 and M-4, where larger cobble supports thick stands of the submersed aquatic macrophyte, *Podostemum ceratophyllum*. No macrophytes are found at M-5, where the dominant habitat types are pebble–gravel runs and depositional areas. Catchment area at midorder stations ranges from 100 to 1000 ha (Houston 1994; Swank and Bolstad 1994).

The river reach includes sixth- and seventh-order sections of the Little Tennessee River. Channel widths vary from 20 to 100 m; catchment area ranges from 10^4 to 10^5 ha. Canopy cover is minimal and extensive stands of *P. ceratophyllum* cover bedrock and cobble substrata. Annual degree-days range from ~4700 to 5100 (Table 1), and water depths generally vary between 0.5 and 1 m but can approach ~1.5 m in pooled (depositional) areas. In the upper 20 km of this reach, streambed slope is minimal and the unbraided river channel meanders through an extensive alluvial floodplain valley (station R-1 lies within this section). Slope becomes steeper in the lower 23 km of the reach (which includes stations R-2 and R-3), and the channel straightens with little floodplain development. Cobble riffles and depositional areas are dominant habitat types at R-1, but as slope increases downstream, bedrock outcrops become prevalent. Cobble riffles are absent at station R-3 (Table 1).

The upper five stations (S-1 through M-2) lie within the boundaries of Coweeta Hydrologic Laboratory (CHL) and are relatively unaffected by anthropogenic influences. Residential and agricultural land use is minimal downstream from CHL (stations M-3 through M-5); over 91% of the Coweeta basin still remains in contiguous forested areas (Swank and Bolstad 1994), and for much of its length, Coweeta Creek is flanked with a wooded riparian zone. The greatest potential for anthropogenic influence exists in the upper, alluvial section of the Little Tennessee River (station R-1). Row-crop agriculture and grazing is practiced on the floodplain and the river passes through the municipality of Franklin, N.C., where a small hydroelectric dam is located. Station R-1 was established upstream of Franklin in a stream segment flanked with riparian buffer strips between the river and row-cropped areas to minimize impacts. Sampling stations in the lower segment of the Little Tennessee (R-2 and R-3) are situated well downstream of Franklin (>3.5 km) and within the Nantahala National Forest. This segment is heavily forested with little residential or agricultural land use.

Materials and methods

Field procedures

Instead of fixed points, sampling stations established along the continuum are discrete stream segments that vary in length from ~60 m in the small-order reach to over 100 m in the river reach. Mesoscale habitats within each segment were visually identified as bedrock outcrops, cobble riffles, pebble–gravel runs, or depositional areas. To determine proportional availability of habitats at each sampling station, at least 100 random measurements of substratum particle size were taken in August of 1991 throughout each stream segment (over 300 measurements were taken at river stations). Occurrence frequency of particle size-classes were used as an indication of relative avail-

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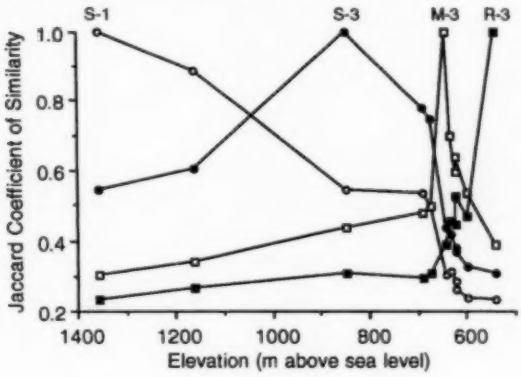
Fig. 2. Congruity curves based on taxonomic composition for 4 of 11 sampling stations along the longitudinal gradient. Curves are based on the Jaccard coefficient of similarity, and zones of rapid faunal replacement are denoted by steep curve slopes. Site codes are given in Fig. 1.



ability of each habitat. Specifically, particle size >256 mm represented bedrock outcrops; 65–255 mm, cobble riffles; 2–64 mm, pebble–gravel runs; <2 mm, depositional areas. All four habitats were never adequately represented at a given station; thus, two or three of the most prevalent habitats at each station were sampled (see Table 1).

Monthly macroinvertebrate collections were taken in summer (July, August, and September 1991) and seasonal samples in autumn (November 1991), winter (January 1992), and spring (May 1992) for a total of seven collection dates. On each date, three replicate samples were taken from prevalent habitats at each sampling station. Specific collecting techniques depended upon reach size and type of habitat sampled. In all cases, 250-µm mesh catchnets were used. Bedrock outcrops in small-stream and midorder reaches were sampled by scraping and brushing material from 100-cm^2 rockface areas into catchnets (Marcilio 1993; Houston 1994). Deep water prohibited this approach at river stations; instead, we used a modified T-sampler with an effective sampling area of 103 cm^2 (English 1987). Cobble and pebble–gravel habitats were generally sampled with a 930-cm^2 Surber sampler to a depth of approximately 10 cm (Marcilio 1993; Houston 1994). In the river reach, we sampled deep-water cobble by placing a catchnet on the bottom and dislodging an upstream stone. Material was scraped and brushed from the stone into the catchnet and cobble dimensions were measured to determine surface area. To sample depositional areas at small-stream stations, a 400-cm^2 corer was driven into the substratum and material excavated to a depth of approximately 10 cm, placed into a bucket, repeatedly elutriated, and washed through a 250-µm mesh net (Marcilio 1993; Houston 1994). In depositional areas in midorder and river reaches, an Eckman dredge was used to collect 232-cm^2 samples to a depth of approximately 10 cm; these samples were also elutriated in the field. All samples were preserved in the field with 5–10% formalin containing Phloxine-B dye to aid in sample processing.

Laboratory procedures

Samples were washed through 1-mm and 250-µm nested sieves; material retained on the 1-mm sieve was sorted at 15× magnification and all macroinvertebrates removed. Using a sample splitter (Waters 1969), a 1/2, 1/8, or 1/64 subsample was taken from material retained on the 250-mm sieve, and invertebrates were removed under 30× magnification.

Aquatic plants (mosses or *P. ceratophyllum*) were separated from bedrock and cobble samples and thoroughly washed to remove any trapped material. Plants were dried at 60°C for 5–7 days, weighed, ashed at 550°C for 12 h, then reweighed to determine ash-free dry mass (AFDM). Organic matter from depositional samples retained on sieves was treated similarly to determine AFDM of benthic organic matter (BOM).

Macroinvertebrate identifications were generally made to genus for insects, exceptions being dipteran families of Chironomidae (identified as Tanypodinae and non-Tanypodinae), Ceratopogonidae, and Simuliidae, and the plecopteran family Peltoperlidae. Non-insects were identified to class or order. For all taxa, levels of identification were sufficient to allow assignment of functional feeding-group designations, based on guidelines in Merritt and Cummins (1984) or Huryn and Wallace (1987*a*). Crayfish (Decapoda) were designated as 50% shredders, 25% collector–gatherers, and 25% predators (Huryn and Wallace 1987b); *Micrasema* spp. (Trichoptera: Brachycentridae) were assigned as 50% collector-filterers and 50% shredders (Merritt and Cummins 1984).

Macroinvertebrates were counted, measured (total body length to the nearest millimetre), and individuals per sample standardized to individuals per square metre to adjust for differing areas sampled by each collection technique. Individual biomass was calculated as milligrams AFDM from predetermined length–weight regression equations for each taxon. Biomass values (milligrams AFDM per square metre) and abundances (individuals per square metre) are reported as annual means.

Analysis of functional composition along the gradient

Functional composition along the continuum was characterized both in terms of habitat-specific and habitat-weighted biomass. Habitat-specific biomass was defined as milligrams AFDM per square metre of given habitat type. To estimate habitat-weighted biomass, habitat-specific biomass values were multiplied by proportional habitat availability and products were summed for individual sampling stations.

Statistical analyses

To determine patterns of faunal replacement along the elevational gradient, we used the Jaccard coefficient to generate congruity curves. This coefficient ranges from 0 (no similar taxa) to 1 (no dissimilar taxa) and accounts for presence or absence of taxa rather than relative density (Brower and Zar 1984). Each station was used in turn as a reference (a similarity coefficient versus itself of 1) and coefficients were determined against the other 10 stations. These points were then plotted across the elevational gradient to produce a congruity curve; areas along the curve with steep slopes (i.e., abrupt changes in the similarity coefficient) are indicative of stream reaches with rapid faunal replacement (Allan 1975*a*, 1975*b*); curves in this study were also plotted against an elevational rather than stream size gradient to match the procedure used by Allan (1975*a*, 1975*b*). For graphical clarity, only 4 congruity curves were plotted (Fig. 2), but trends were consistent along the gradient for all 11 curves.

For macroinvertebrate abundances, biomass values, and functional composition along the continuum, linear regressions were computed using catchment area (hectares of basin area upstream of the sampling station) as a measure of stream size. The stream-size gradient was used in preference to the elevational gradient as RCC predictions also address stream size (Vannote et al. 1980). Biomass data collected from Ball Creek headwaters by Huryn and Wallace (1987*a*) were incorporated into the regression computations to increase station numbers. Percentages of functional group composition based on mean annual abundance, and biomass estimates were first arcsine transformed prior to calculating regression equations (Zar 1984).

Results

Taxonomic composition

A total of 102 insect genera from 7 orders and 13 families, orders, or classes of various non-insect groups were collected

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Table 2. Macroinvertebrate taxon richness at sampling stations along the longitudinal gradient.

Sampling station	S–1	S–2	S–3	M–1	M–2	M–3	M–4	M–5	R–1	R–2	R–3
By sampling station	61	61	81	89	93	106	87	70	73	67	44
By habitat type											
Bedrock outcrops	40	39	50	49	61					41	34
Cobble riffles	56	50	64	71	72	99	77		67	48	
Depositional areas	44	41	40	44	51	48	45	38	26	41	21
Pebble–gravel								58			

Note: Total taxa collected at each station and for each habitat are listed. Station codes are given in Fig. 1.

along the longitudinal gradient. Common taxa collected (those that accounted for more than 1% of functional group biomass) are listed by functional feeding group and sampling station in Appendix Tables A1–A5.

Biotic diversity was greatest in the midorder reach (Table 2). The largest number of taxa collected was 106 at station M-3, and richness progressively declined with increasing or decreasing elevation and stream size. On average, diversity was highest on cobble riffles, followed by bedrock outcrops, pebble–gravel runs, and depositional areas. Longitudinal trends in taxon richness on individual habitat types (i.e., bedrock, cobble, and depositional areas) also indicated greatest diversity at midorder stations M-2 and M-3. No trends could be inferred for pebble–gravel habitat as it was encountered at only one sampling station (Table 2).

The midorder reach was also the zone of greatest faunal replacement along the elevational gradient; the steepest slopes in the 11 congruity curves occurred over a short stream segment between stations M-2 and M-3 (Fig. 2). Congruity curves also indicated that faunal replacement was again high between the lower two river stations (R-2 and R-3).

Abundance

Macroinvertebrate abundances were generally greatest in cobble–riffle habitats, followed by bedrock outcrops, pebble–gravel runs, and depositional areas. Habitat-specific abundances changed with stream size along the gradient; invertebrate numbers increased downstream on bedrock outcrops and cobble riffles (Figs. 3*a* and 3*b*) and decreased in depositional areas (Fig. 3*c*). Benthic abundances on bedrock outcrops and cobble riffles were significantly correlated with aquatic plant standing crops ($r = 0.97$, $p < 0.001$, $n = 16$); abundances in depositional habitats were significantly correlated with standing crops of BOM ($r = 0.84$, $p < 0.001$, $n = 12$).

Regardless of habitat and stream size, collector–gatherers consistently provided the primary contributions to functional feeding-group abundance throughout the gradient. Gatherers accounted for 34–84% of abundance on bedrock outcrops, 50–84% in cobble riffles, 53% in pebble–gravel habitat, and 82–93% in depositional areas. With the exception of the downstream river station (R-3) where collector–filterers in bedrock habitat accounted for 42% of total abundance, gatherers were the only individual functional feeding group to provide more than 33% to total abundance. Subsequently, gatherer dominance was also reflected in estimates of habitat-weighted, total abundances along the continuum (Table 3). Gatherers alone accounted for over half of the total benthic abundance throughout the gradient, again with the only exception being station R-3.

Biomass

Similar to abundance estimates, cobble riffles generally supported highest annual mean biomass, followed by bedrock outcrops, pebble–gravel runs, and depositional areas. Also similar to abundances, habitat-specific biomass values associated with bedrock outcrops (Fig. 3*d*) and cobble riffles (Fig. 3*e*) increased with stream size along the gradient, and benthic biomass decreased in depositional areas (Fig. 3*f*). Macroinvertebrate biomass was significantly correlated with aquatic plant biomass on bedrock outcrop and cobble-riffle habitats ($r = 0.96$, $p < 0.001$, $n = 16$); biomass in depositional habitats was significantly correlated to standing crops of BOM ($r = 0.80$, $p < 0.001$, $n = 12$).

Although longitudinal trends in total benthic biomass were similar to trends for total abundance, functional feeding-group composition based on biomass differed significantly from abundance-based composition at all stations and in all sampled habitats (chi-square goodness of fit, $p < 0.001$). Abundance-based results were consistently dominated by gatherers, while biomass-based functional feeding-group composition changed substantially with both habitat and stream size throughout the continuum. On bedrock outcrops, collector–filterers accounted for major portions of benthic biomass along the continuum, especially in small-stream and river reaches (Fig. 4, first column). Percentages of collector–gatherer and scrapers on bedrock peaked in the midorder reach, while shredders and predators decreased with increasing stream size. In cobble-riffle habitats, scraper contributions were again highest in midorder reaches and shredders and predators again decreased downstream (Fig. 4, second column). Percentages of collector–filterer biomass were low in headwater cobble riffles and progressively increased with stream size. No longitudinal trend was evident for collector–gatherers in cobble riffles. In depositional areas, no trends were evident for scraper biomass along the continuum (Fig. 4, third column). Collector–gatherer and collector–filterer biomass both increased downstream, and as in other habitats, contributions of shredders and predators decreased with stream size in depositional areas.

Estimates of mean annual habitat-weighted biomass along the continuum ranged from 0.9 to 18.2 g AFDM·m^{-2}, and as with habitat-specific biomass, functional feeding-group composition of the habitat-weighted benthic community changed with stream size along the continuum (Table 4). The longitudinal trend for total benthic biomass was strongly correlated ($r = 0.91$, $p < 0.001$, $n = 12$) to stream size (Fig. 5*a*). Similar trends occurred for four of five functional groups as scraper, collector–gatherer, collector–filterer, and predator biomass increased with stream size along the continuum (Figs. 5*b*–5*e*). No discernible trends were apparent for habitat-weighted shredder biomass (Fig. 5*f*).

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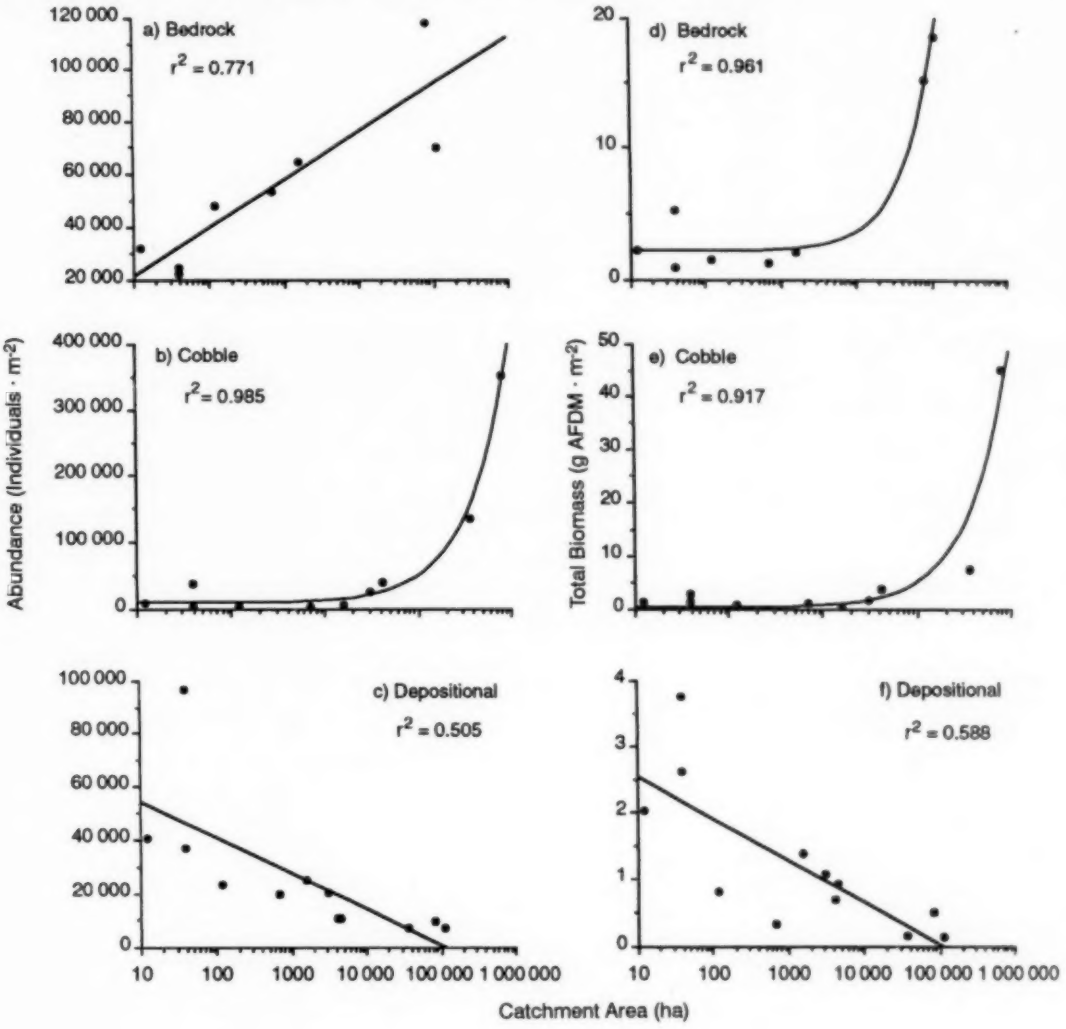
Fig. 3. Longitudinal trends in macroinvertebrate abundances (*a–c*) and biomass (*d–f*) for bedrock outcrop, cobble riffle, and depositional habitats along the Ball Creek – Coweeta Creek – Little Tennessee River continuum.



Percent contribution of scrapers to total habitat-weighted benthic biomass was greatest in the midorder reach (Fig. 6*a*), but relative to other functional groups, scraper biomass was consistently low and made up less than 30% of total habitat-weighted biomass even in the midorder reach (Table 4). Contributions of shredders were highest in the small-stream reach and declined downstream (Fig. 6*b*). Collector–filterer biomass increased substantially with stream size (Fig. 6*c*) and accounted for over 55% of functional feeding-group composition at the two lowest stations (Table 4). Collector–gatherers showed no trend relative to the continuum (Fig. 6*d*), and gatherer biomass accounted for 17–30% of functional composition except at station M-5 where it exceeded 50% (Table 4). Habitat-weighted biomass of benthic predators steadily decreased downstream along the gradient (Fig. 6*e*).

Discussion

Taxon composition

Higher taxon richness and rapid faunal replacement in the midorder reach of this southern Appalachian continuum shows the influence of localized patterns of stream geomorphology on taxonomic composition (Statzner and Higler 1985, 1986). The midorder reach represents the zone of change between the higher elevation, steeply sloped mountain streams and the lower elevation, shallow-sloped alluvial river reach. As a transitional point between these two geomorphic conditions, the midorder reach is an ecotone, serving as either the downstream or upstream limit of ecological tolerance for many taxa (Statzner and Higler 1986); thus, rapid faunal replacement occurs over a relatively short stream reach (Fig. 2). Because of

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Table 3. Macroinvertebrate abundances by functional group along the Ball Creek – Coweeta Creek – Little Tennessee River continuum.

	Abundance (no.·m^{-2}, ×10^3)						Percent				
	SC	SH	CG	CF	PR	Total	SC	SH	CG	CF	PR
S–1	0.4	1.5	18.6	1.1	3.1	24.7	1.6	6.1	75.3	4.4	12.6
S–2	0.6	0.9	9.2	0.8	1.3	12.8	4.7	7.0	71.9	6.2	10.2
S–3	0.6	0.7	14.2	0.8	2.1	18.4	3.3	3.8	77.2	4.3	11.4
H&W	0.7	2.2	42.4	0.2	3.4	48.9	1.4	4.5	86.7	0.4	7.0
M–1	1.0	0.6	10.8	0.8	2.0	15.2	6.6	3.9	71.0	5.3	13.2
M–2	1.7	0.5	9.2	0.7	1.3	13.4	12.7	3.7	68.6	5.2	9.7
M–3	6.8	1.3	29.5	5.8	5.0	48.4	14.0	2.7	61.0	12.0	10.3
M–4	5.0	0.4	16.2	4.8	3.1	29.5	16.9	1.4	54.9	16.3	10.5
M–5	1.0	0.1	4.2	0.7	0.5	6.5	15.4	1.5	64.7	10.7	7.7
R–1	1.3	0.6	25.5	12.8	2.3	42.5	3.0	1.4	60.0	30.1	5.4
R–2	11.2	2.6	75.3	42.2	12.4	143.7	7.8	1.8	52.4	29.4	8.6
R–3	9.2	0.3	19.1	21.1	2.9	52.6	17.5	0.6	36.3	40.1	5.5

Note: Abundances are habitat weighted by sampling station and reported as mean annual number of individuals per 10^3 m^2 (left-hand columns) and as percentages of total abundance. H&W, data collected from the Ball Creek headwaters by Huryn and Wallace (1987*a*); SC, scrapers; SH, shredders; CG, collector–gatherers; CF, collector–filterers; PR, predators. Station codes are given in Fig. 1.

a certain degree of overlap among taxa associated solely with upstream or downstream geomorphic conditions, an edge effect accounts for the higher biotic diversity observed in the midorder reach (Table 2).

Habitat-specific abundance and biomass

Macroinvertebrate abundances and biomass were greatest in cobble riffles relative to bedrock, gravel–pebble, and depositional areas. Ward (1975) reported a similar relationship between substratum type and benthic densities in North St. Vrain Creek (Colorado). Minshall (1984) suggested increased invertebrate densities associated with cobble substrata are likely due to greater substratum particle-size heterogeneity of cobble relative to sand, gravel, or bedrock. On bedrock outcrops and cobble riffles, benthic abundance and biomass were significantly correlated with aquatic plant standing crops, which reinforces the theory that macrophyte substrates generally support higher macroinvertebrate densities than mineral substrates (Minshall 1984). In depositional areas, which contain no aquatic plants, macroinvertebrate densities were significantly correlated with standing stocks of BOM. Functional feeding-group composition in depositional habitats was dominated by either gatherers or shredders (Fig. 4, third column), both of which utilize BOM as a food resource (Merritt and Cummins 1984; Wallace et al. 1992) and suggest benthic densities in depositional habitats are influenced by food resource availability along the gradient.

Although longitudinal trends of total benthic densities were similar for abundance and biomass data (see Fig. 3), functional feeding-group composition was very different between abundance and biomass-based estimates. Small-bodied taxa, such as non-Tanypodinae chironomids, oligochaetes, and copepods, were ubiquitous in all benthic samples, regardless of habitat or stream size sampled. Because these taxa were primarily designated as collector–gatherers, abundance-based functional feeding-group composition was consistently dominated by collector–gatherers throughout the continuum (Table 3). These taxa, however, account for relatively little of total benthic biomass. Subsequently, community composition based on functional feeding-group biomass changed both with habitat and stream size along the continuum (Fig. 4).

Functional feeding-group composition of the benthic community appears to be related to the physical characteristics specific to individual habitats. Bedrock outcrops of the southern Appalachians are characterized by low substrate roughness and rapid current velocity relative to other habitat types (Huryn and Wallace 1987*a*; Wallace et al. 1992). Collector–filterers are well suited to exploit these conditions, using holdfast structures and refugia to maintain position while capturing entrained food resources with catchnets or modified appendages (Wallace and Merritt 1980). The presence of aquatic plants (mosses in the headwaters and *P. ceratophyllum* downstream) further facilitates filterer colonization by providing abundant refugia and catchnet attachment sites. Thus, filterers were consistently a major component of benthic community composition on bedrock outcrops throughout the continuum, accounting for 20–65% of total benthic biomass (Fig. 4, first column). Similarly, contributions of filterers to benthic biomass in cobble riffles increased with stream size (Fig. 4, second column). As the stream widens and canopy cover diminishes, increased light availability allows for colonization of cobble substratum by *P. ceratophyllum*, thereby providing more attachment sites for filtering macroinvertebrates.

Scraper contributions were never a dominant component of benthic community composition in any of the habitats; however, scraper contributions were greatest on bedrock and cobble of the midorder reach than elsewhere along the gradient (Fig. 4). Periphyton is an important food resource for scrapers (Merritt and Cummins 1984), and periphyton production is likely to increase in the midorder reach as stream width increases, enhancing light availability to the stream. Furthermore, McAuliffe (1983) demonstrated that moss and macrophyte biomass favors colonization of filterers over scrapers, which could account for subsequent downstream reduction of scraper contributions to benthic biomass as standing crops of *P. ceratophyllum* increased with stream size. Scraper biomass in depositional habitat was not correlated with the longitudinal gradient (Fig. 4, third column), which is not surprising, because fine sand and silt substrata of depositional areas do not support extensive standing crops of periphyton.

Shredders and gatherers comprised the greatest proportions

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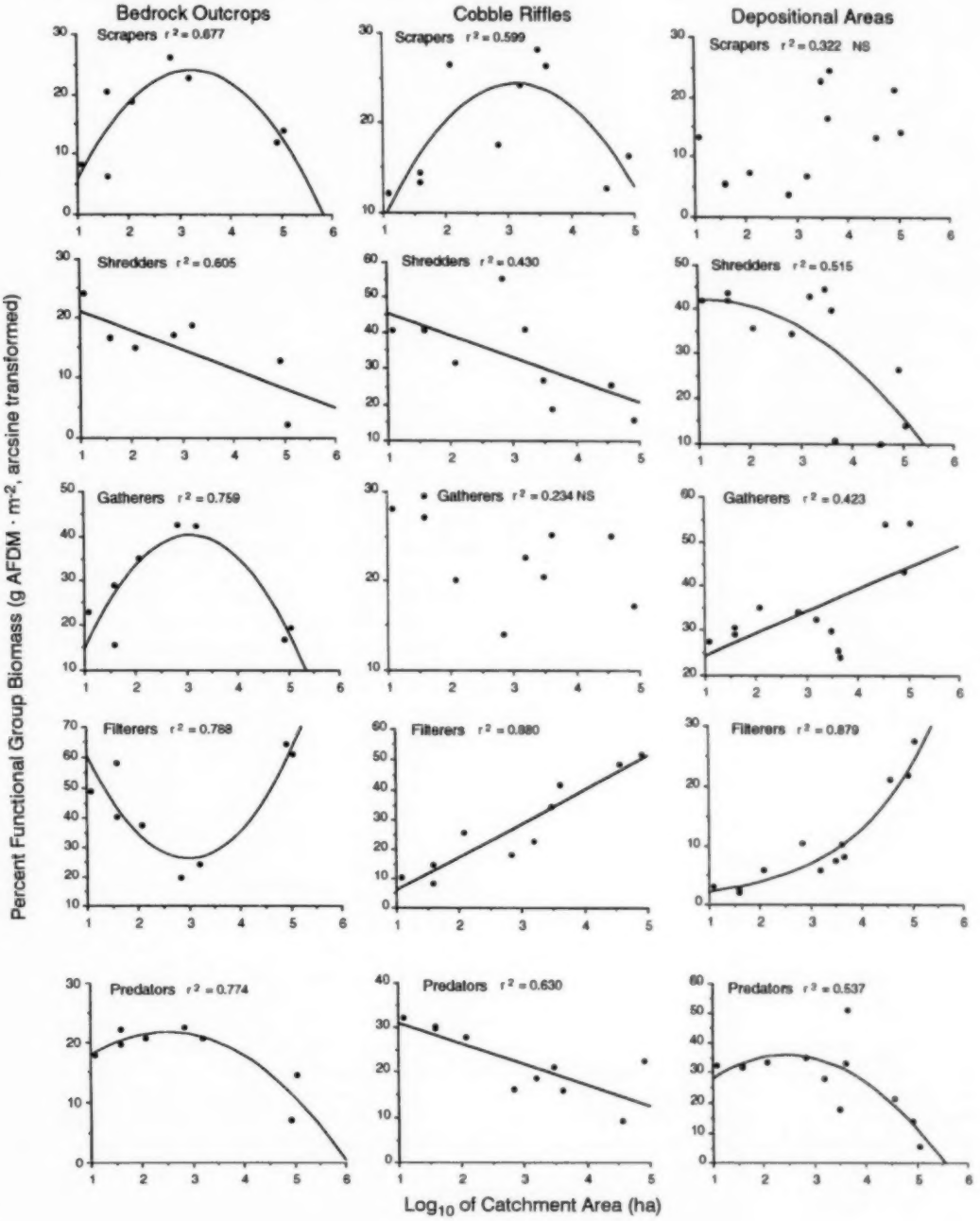
Fig. 4. Relative contributions of functional feeding groups to benthic community structure associated with bedrock outcrop, cobble riffle, and depositional habitats along the Ball Creek – Coweeta Creek – Little Tennessee River continuum. Contributions are expressed as percentages of total habitat-specific biomass at each sampling station, arcsine transformed prior to plotting to obtain a nearly normal distribution (Zar 1984).


Bedrock Outcrops
Cobble Riffles
Depositional Areas
Scrapers r² = 0.677
Scrapers r² = 0.599
Scrapers r² = 0.322 NS
Shredders r² = 0.605
Shredders r² = 0.430
Shredders r² = 0.515
Gatherers r² = 0.759
Gatherers r² = 0.234 NS
Gatherers r² = 0.423
Filterers r² = 0.788
Filterers r² = 0.880
Filterers r² = 0.879
Predators r² = 0.774
Predators r² = 0.630
Predators r² = 0.537
Percent Functional Group Biomass (g AFDM · m⁻², arcsine transformed)
Log10 of Catchment Area (ha)

of benthic biomass in depositional areas, with shredder contributions decreasing with stream size and the gatherer component increasing (Fig. 4, third column). Depositional areas of small-stream reaches in the southern Appalachians are usually associated with debris dams that are relatively stable and accumulate a large proportion of coarse particulate organic matter

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Table 4. Macroinvertebrate biomass by functional feeding-group along the Ball Creek – Coweeta Creek – Little Tennessee River continuum.

	Abundance (no.·m^{-2}, ×10^3)						Percent				
	SC	SH	CG	CF	PR	Total	SC	SH	CG	CF	PR
S–1	0.06	0.50	0.35	0.80	0.31	2.01	2.8	24.8	17.3	39.6	15.4
S–2	0.08	0.66	0.37	0.72	0.45	2.27	3.4	29.1	16.2	31.5	19.9
H&W	0.12	1.15	0.65	0.11	0.69	2.73	4.4	42.1	24.0	4.2	25.2
S–3	0.13	0.21	0.22	0.21	0.19	0.96	13.3	21.7	22.9	22.0	20.2
M–1	0.13	0.68	0.17	0.13	0.12	1.23	11.0	55.0	14.1	10.2	9.7
M–2	0.12	0.36	0.20	0.11	0.12	0.92	13.0	39.5	22.0	12.0	13.5
M–3	0.41	0.52	0.19	0.42	0.28	1.81	22.5	28.6	10.4	23.1	15.4
M–4	0.50	0.36	0.49	1.07	0.27	2.69	18.6	13.4	18.1	40.0	9.9
M–5	0.22	0.02	0.93	0.08	0.25	1.50	14.9	1.2	62.2	5.1	16.5
R–1	0.12	0.56	0.48	1.18	0.07	2.41	5.1	23.1	20.0	49.0	2.7
R–2	1.25	1.21	1.62	12.23	1.89	18.19	6.8	6.7	8.9	67.2	10.4
R–3	0.79	0.02	1.52	10.33	0.85	13.51	5.9	0.2	11.2	76.5	6.3

Note: Biomass measurements are habitat-weighted by sampling station and reported as mean annual biomass (g AFDM·m^{-2}) in the left-hand columns and as percentages of total biomass in the right-hand columns. H&W, data collected from the Ball Creek headwaters by Huryn and Wallace (1987*a*); SC, scrapers; SH, shredders; CG, collector–gatherers; CF, collector–filterers; PR, predators. Station codes are given in Fig. 1.

(Wallace et al. 1992), a primary food resource for shredder macroinvertebrates. As stream size increases, depositional areas are less associated with debris dams and exist more as sand–silt areas subject to frequent scouring that collect fine particulate organic matter (FPOM). These characteristics are conducive to collector–gatherer taxa, which utilize FPOM as a food resource and generally have multivoltine life histories that allow rapid recolonization of disturbed habitats (Lugthart and Wallace 1992). Increased contributions of collector–filterer biomass with stream size was attributable to downstream populations of taxa rarely found upstream (i.e., sphaeriid clams and ephemerid mayflies), which can successfully utilize habitats of finer grained substrata.

In all three habitats, predator contributions to total benthic biomass decreased with stream size along the gradient (Fig. 4, last row), but caution must be exercised in interpreting these results. These estimates of predator biomass address only the macroinvertebrate community and do not account for vertebrate predators such as fishes or salamanders, the former of which were absent from stations S-1 and S-2, but taxon richness of fishes along this continuum steadily increases with stream size (see Freeman et al. 1988; McLarney 1990). Furthermore, diets of hydropsychid caddisflies, which make up the majority of collector–filterer biomass in the lower portion of the gradient, consist largely of animal material (Ross and Wallace 1982) and, hence, are functioning as both filterer and invertebrate predator.

Assessments of functional feeding-group composition of benthic communities are profoundly influenced by which parameter is measured (abundance or biomass), the habitat sampled, and the relative position of sample collection along the stream size continuum. One cannot assume abundance and biomass-based interpretations of benthic community structure are interchangeable or that sampling a single habitat can adequately represent benthic community composition along an entire stream gradient. Parameters measured should match the question asked (e.g., using biomass measurements to test RCC hypotheses), and sampling regimes should be designed to accommodate localized changes in the relative availability of benthic macroinvertebrate habitats.

Habitat-weighted biomass

Along the Ball Creek – Coweeta Creek – Little Tennessee River continuum, habitat-weighted biomass of total benthos and four of five functional feeding groups were linearly and significantly correlated with catchment size (Figs. 5*a*–5*e*). It is important to note that this relationship is due in large part to the high biomass values at the downstream stations; little correlation would exist if only smaller stream stations (i.e., <1000 ha) had been sampled. These results underscore the importance of incorporating larger order stream reaches into analyses of longitudinal trends along stream continua.

No longitudinal trend was apparent for habitat-weighted shredder biomass (Fig. 5*f*), which may be over-represented at lower midorder and river stations due to ambiguities in assigning taxa to this functional feeding group. Although *Pteronarcys* is primarily known as a detrital leaf shredder (McDiffit 1970), it can also be an opportunistic feeder, often functioning as a scraper in streams with limited inputs of allochthonous material (Freilich 1991). Debris dams and leaf packs were rare to nonexistent at downstream stations, yet *Pteronarcys* was often present and provided a substantial contribution to shredder biomass (see Table A2). *Pteronarcys* at stations R-1 through R-3 use food resources other than detrital leaf material to subsidize dietary needs (G.R. Plague, J.B. Wallace, and J.W. Grubaugh, unpublished data). In addition, half of *Micrasema* biomass was assigned to the shredder group, and this trichopteran also contributed substantially to downstream shredder biomass (see Table A2). *Micrasema*, however, is a shredder–herbivore rather than a shredder–detritivore and, therefore, unlike other shredders in this system. Placing these taxa into the detrital shredder group may overestimate habitat-weighted, shredder–detritivore biomass at downstream sampling stations. In fact, if these taxa are omitted from the shredder functional feeding group, habitat-weighted shredder biomass decreases downstream and is negatively correlated with the stream size gradient ($r = -0.67$, $p < 0.05$, $n = 12$).

Longitudinal trends predicted by the RCC were based on relative dominance (as biomass) of functional feeding-group composition along a stream continuum (Vannote et al. 1980; Minshall et al. 1985). Along the Ball Creek – Coweeta Creek

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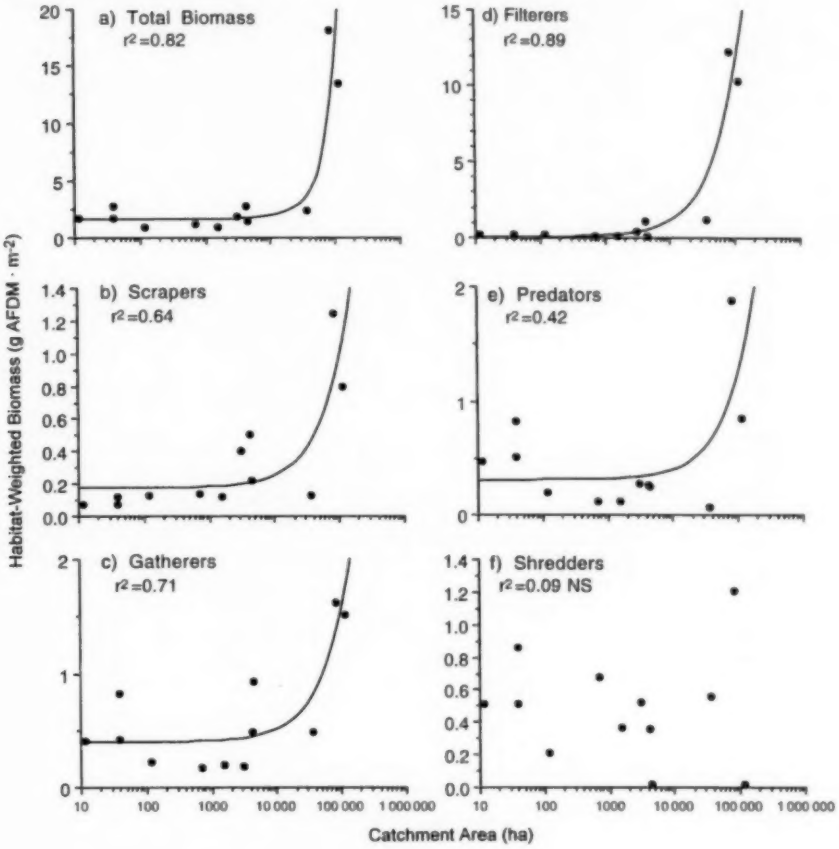
Fig. 5. Longitudinal trends in habitat-weighted biomass (g AFDM·m^{-2}) for functional feeding groups along the Ball Creek – Coweeta Creek – Little Tennessee River continuum. Individual figures indicate (*a*) total biomass and (*b*) scraper, (*c*) collector–gatherer, (*d*) collector–filterer, (*e*) predator, and (*f*) shredder biomass.



– Little Tennessee River continuum, relative patterns in functional feeding-group composition generally supported predictions of the RCC (Fig. 6). Scraper proportions were low in headwater and river reaches and high in the midorder reach (Fig. 6*a*). Shredder contributions (with or without *Micrasema* and *Pteronarcys* biomass) were greatest at small-stream and upper midorder reach sampling stations and diminished downstream with increasing stream size (Fig. 6*b*). Conversely, relative dominance of collector–filterer biomass increased along the stream-size gradient (Fig. 6*c*).

The localized influences of relative habitat availability on functional feeding-group composition were also apparent along the Ball Creek – Coweeta Creek – Little Tennessee River continuum. Collector–filterers were a substantial component of the benthic community in the smaller stream reach, accounting for over 30% of total biomass at three of four upstream stations (Fig. 6*c*); the RCC predictions indicated filterers should be rare in headwater streams (Minshall et al. 1985). High proportions of bedrock-outcrop habitat throughout Ball Creek provided ample habitat and greater densities of filterers in the small-stream reach. Collector–gatherers showed no trends with regard to the gradient but accounted for over 50% of habitat-weighted biomass at one midorder station, M-5 (Fig. 6*d*). Gravel–pebble runs and depositional areas prevailed at M-5, and substrata favored by filterers (i.e., bedrock or cobble with vegetation) were absent (Table 1). Thus, a lack of suitable substrata at M-5 potentially reduced the relative contribution of filterers, and smaller particle-size distributions favored small-bodied gatherers.

The relative contribution of invertebrate predators to community structure decreased with increasing stream size (Fig. 6*e*), which does not support the RCC prediction that predator contributions should remain constant throughout the continuum (Vannote et al. 1980). Again, predatory collector–filters are not considered part of this functional group and fish populations, which did not occur in the small-stream reach but became increasingly important as stream size increased, were excluded from this study. Reduced contributions of macroinvertebrate predators to habitat-weighted benthic biomass also may suggest competitive or predatory influences of fishes in downstream reaches.

Surprisingly few studies have used measures of functional

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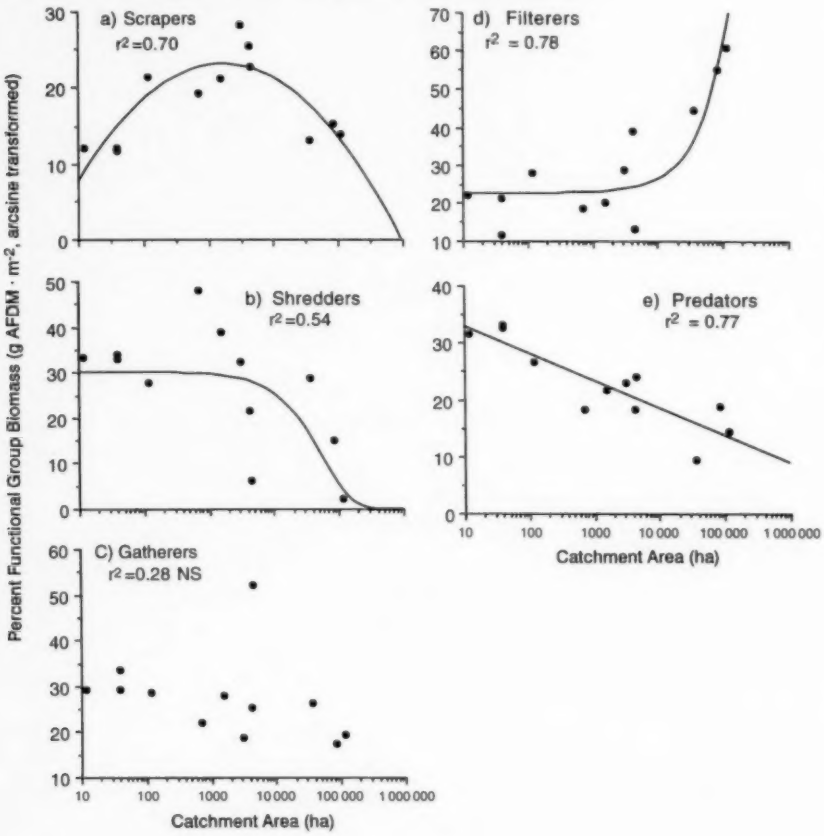
Fig. 6. Relative contributions of functional feeding-groups to habitat-weighted benthic community structure along the Ball Creek – Coweeta Creek – Little Tennessee River continuum. Contributions are expressed as percentages of total habitat-weighted biomass at each sampling station, arcsine transformed prior to plotting to obtain a nearly normal distribution (Zar 1984). Individual figures indicate the contributions of (*a*) scrapers, (*b*) shredders, (*c*) collector–gatherers, (*d*) collector–filterers, and (*e*) predators to benthic community structure.



feeding-group biomass of benthic communities along stream continua. Ward (1986) examined longitudinal changes in taxonomic biomass along the St. Vrain River system, but used abundance-based estimates of relative composition to characterize community functional structure along the continuum. Naiman et al. (1987) used biomass-based measures of benthic diversity to compare community structure in streams of different sizes along the Gulf of St. Lawrence (Quebec, Canada), but these streams were not part of a single continuum nor was functional composition described.

Brussock and Brown (1991) incorporated biomass-based estimates of functional composition to examine community structure in first- through fifth-order reaches of the Illinois River (Arkansas, U.S.A.). Geomorphic structure of this system is characterized by low streambed relief, intermittent flow in the headwaters, and a gravel riffle–pool sequence of habitat availability. Biomass-based functional composition was dominated by collector–gatherers throughout the Illinois River continuum and, hence, showed little agreement with longitudinal functional structure predicted by the RCC.

With qualifications, the Ball Creek – Coweeta Creek – Little Tennessee River continuum fits the predictions of the RCC. This is not surprising, given the physical template of the longitudinal gradient. Brussock and Brown (1991) noted benthic macroinvertebrate data that best fit the predictions of the RCC come from continua with streambed slopes greater than 10% in the headwaters, which is a feature of this southern Appalachian continuum. Furthermore, Ward (1986) pointed out that the RCC was derived primarily from forested streams of eastern North America. Our system has heavily forested headwater reaches with steep streambed gradients and high streambed roughness; thermal and hydrologic regimes are strongly influenced by groundwater, and canopy cover decreases with stream order. These physical and biological features contribute to strong elevational and longitudinal changes with resultant effects on community structure.

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Appendix 1. Habitat-weighted biomass (mg AFDM·m^{-2}) of dominant taxa for each functional group and sampling station along the Ball Creek – Coweeta Creek – Little Tennessee River continuum.

Table A1. Scrapers.

Taxon	Insect order[a]	S-1	S-2	S-3	M-1	M-2	M-3	M-4	M-5	R-1	R-2	R-3
Promoresia	C			2	15	7	8	230	14	23	649	314
Baetis	E	1	6	11	26	46	117	125	34	59	288	467
Stenonema	E	49	38	57	46	11	31	16	14	9	101	1
Epeorus	E	2	12	45	20	28	112	4	8	4	2	
Optioservus	C	1	3	6	3	6	40	46	34	<1	5	
Ancylidae	—						15	17	15	<1	94	3
Hydroptila	T					2	2	13	6	<1	2	75
Elimia	—						29	30	29	7	1	
Macronychus	C						1	1	37	2	10	
Goera	T						4	6	28	3		
Others		18	20	6	22	19	38	20	10	14	21	6
Total scraper biomass		71	79	127	134	119	407	499	223	124	1245	792[a]

Note: Insect orders are: C, Coleoptera; D, Diptera; E, Ephemeroptera; M, Megaloptera; O, Odonata; P, Plecoptera; T, Trichoptera.

Table A2. Shredders.

Taxon	Insect order	S–1	S–2	S–3	M–1	M–2	M–3	M–4	M–5	R–1	R–2	R–3
Pteronarcys	P			<1	571	224	327	136		391	731	1
Decapoda	—	356	526	85	1	81		101				
Peltoperlidae	P	96	138	89	84	19	30	7				11
Tipula	D	14	1		2		60	29		66	155	
Micrasema	T		1	2	2	<1	19	46	3	28	227	4
Pycnopsyche	T	80	2	10	3	21	67	34	2		2	
Taeniopteryx	P				2	2	5	3	11	66	54	2
Leuctra	P	49	14	11	8	14	4	2	<1	<1		
Others		39	7	10	2	1	4	3	3	6	40	3
Total shredder biomass		634	689	208	675	362	517	360	18	557	1210	21

Note: See Table A1 for insect orders.

Table A3. Collector–gatherers.

Taxon	Insect order	S–1	S–2	S–3	M–1	M–2	M–3	M–4	M–5	R–1	R–2	R–3
Chironomidae	D	56	22	49	41	40	68	120	600	177	562	114
Ephemerella	E			19	23	22	7	191	80	174	421	786
Serratella	E	31	22	53	64	28	11	56	1	36	71	415
Oligochaeta	—	37	23	37	11	12	11	20	192	40	416	18
Decapoda	—	178	263	43	1	41		50				
Antocha	D	1	3	6	2	2	14	9	25	48	60	22
Ameletus	E	3	2	<1		2	<1	6		<1		160
Drunella	E			<1	11	23	14	12	<1	2	16	
Amphinemura	P	22	3	4	6	<1						
Others		22	23	8	14	32	63	23	33	3	77	<1
Total gatherer biomass		350	361	219	173	202	189	487	931	480	1623	1517

Note: See Table A1 for insect orders.

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Table A4. Collector–filterers.

Taxon	Insect order	S–1	S–2	S–3	M–1	M–2	M–3	M–4	M–5	R–1	R–2	R–3
Hydropsyche	T				16	47	140	445	40	534	8 429	8 596
Cheumatopsyche	T					<1	14	96	15	331	2 321	1 162
Simuliidae	D	2	<1	9	14	13	48	104	13	265	949	529
Parapsyche	T	208	181	129	9							
Brachycentrus	T			<1	13	22	131	365	<1	20	89	26
Micrasema	T		1	2	2	<1	19	46	3	28	227	4
Others		31	46	71	71	26	67	18	5	3	213	16
Total filterer biomass		241	228	211	125	110	419	1074	77	1180	12 228	10 333

Note: See Table A1 for insect orders.

Table A5. Predators.

Taxon	Insect order	S–1	S–2	S–3	M–1	M–2	M–3	M–4	M–5	R–1	R–2	R–3
Corydalus	M									<1	1420	
Paragnetina	P						154		<1	2		686
Decapoda	—	178	263	43	<1	41		50				
Turbellaria	—	8	4	<1	1	<1	1	1	19	2	253	11
Isoperla	P	21	16	29	24	15	10	28	11	2	13	10
Perlesta	P						<1	<1	2	1	83	104
Rhyacophila	T	28	25	14	13	2	23	20	6	<1		
Malirekus	P	2	33	22	9	24						
Hydracarina	—	2	1	4	3	2	2	6	86	4	19	4
Cordulegaster	O			8	4	4			99			
Acroneuria	P			19	35	8	15	18	<1			1
Hexatoma	D	30	20	20	7	9	16		<1	<1	<1	<1
Ceratopogonidae A	D						3	47	10	13	7	<1
Ceratopogonidae	D	18	13	5	6	2	6	4	<1	2		6
Beloneuria	P	51	15	14			<1		<1			
Hemerdromia	D						<1	1	5	8	27	14
Lanthus	O	15	12				3	32				
Nigronia	M						<1	25		12	10	
Others		63	37	16	15	15	45	34	7	18	55	10
Total predator biomass		416	439	193	119	124	279	267	247	66	1886	847

Note: See Table A1 for insect orders.

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PERSPECTIVES

Metabolic ontogeny of teleost fishes

John R. Post and Julie A. Lee

Abstract: Contrasting models of metabolic ontogeny were tested by reanalyzing literature data on teleost fishes. Metabolic allometry of the early life-history stages of teleosts shows wide variation in mass dependence with slopes ranging from –0.45 to 0.14 for mass-specific metabolic rates. Sufficient data to examine metabolism throughout complete life histories was found for only three species, common carp (*Cyprinus carpio*), rainbow trout (*Oncorhynchus mykiss*), and red sea bream (*Pagrus major*). All three species had biphasic ontogenetic patterns. Early life-history stages had mass-independent or nearly mass independent metabolic rates, and older life-history stages had mass-dependent metabolic rates. The inflection point between metabolic phases differs among species and is not correlated to size at metamorphosis. The biphasic metabolism–mass relationship is correlated with ontogenetic changes in the surface area to volume ratio of respiratory organs. There is sufficient data in the literature to examine ontogenetic patterns in metabolic scope for activity for only rainbow trout. As with routine metabolism, active metabolism shows a biphasic allometry with a similar mass at inflection. We propose a general scheme of metabolic ontogeny for teleost fishes that can serve as a hypothesis for testing.

Résumé : On a vérifié des modèles contrastants de l'ontogénie métabolique en ré-analysant des données sur les téléostéens obtenues dans la littérature. L'allométrie métabolique des premiers stades de vie des téléostéens indique une forte variation de la dépendance sur la masse, les pentes variant de –0,45 à 0,14 pour les taux métaboliques spécifiques à la masse. Les données nécessaires à l'examen du métabolisme pendant toute la vie des organismes n'étaient suffisantes que pour trois espèces, soit la carpe, *Cyprinus carpio*, la truite arc-en-ciel, *Oncorhynchus mykiss*, et le pagre, *Pagrus major*. Ces trois espèces possédaient toutes une histoire ontogénétique biphasique. Les taux métaboliques des premiers stades de vie étaient indépendants ou presque indépendants de la masse. Le point d'inflexion entre les phases métaboliques diffère d'une espèce à l'autre et n'est pas corrélé à la taille au moment de la métamorphose. Il y a une corrélation entre la relation métabolisme biphasique – masse et les changements ontogénétiques du rapport superficie–volume des organes respiratoires. C'est uniquement pour la truite arc-en-ciel que la littérature renferme suffisamment de données pour examiner l'ontogénie dans le contexte du métabolisme nécessaire à l'activité. Tout comme avec le métabolisme de routine, le métabolisme actif présente une allométrie biphasique avec une masse semblable au point d'inflexion. Nous proposons pour l'ontogénie métabolique chez les poissons téléostéens un plan général qui pourrait servir d'hypothèse de vérification.

Introduction

The energetic costs of metabolic processes delimit an animal's scope for growth and scope for activity. Throughout ontogeny, across orders of magnitude in mass, metabolic rates are dominated by mass scaling. In the two or three decades following Winberg's (1956) influential publication, a substantial literature has accrued that describes an allometric relationship between mass-specific metabolic rate and mass with an exponent of –0.2 to –0.4 in fishes (Brett and Groves 1979; Jobling 1994) and many other animals (Schmidt-Nielsen 1984; Reiss 1989). Most of the research on fish has involved juveniles and adults, which includes the majority of the life span but typically covers less than half of the change in body mass.

At the time of Winberg's (1956) publication, there had already been substantial research on ontogenetic patterns of metabolic rates of mammals and a biphasic pattern of metabolic scaling had been identified (Brody 1945). Phase 1 is a period of rapid growth in mass associated with proportional increases in metabolic rate and phase 2 is a period during which metabolic rate increases disproportionately less than the rate of mass change (Fig. 1). More recently, biphasic metabolic ontogeny has been identified in a diversity of vertebrate and invertebrate species (Wieser 1984). Mass exponents described for fish by Winberg (1956) were similar to those of large juvenile and adult mammals in the second phase of their ontogeny. Winberg's model has been accepted as the appropriate model of metabolic scaling for fish by a generation of fish biologists (see reviews by Glass 1969; Fry 1971; Brett and Groves 1979; Wootton 1990). However, in the last decade there has been increasing interest in the physiological and ecological processes involved in the growth, survival, and recruitment of the early life-history stages of fishes. There is evidence that metabolic rates of early life-history stages of some fish species scale isometrically with mass (Kamler et al. 1987; Giguère et al. 1988; Rombough 1988*a*; Oikawa et al. 1991; Kamler 1992; Oikawa and Itazawa 1992*b*).

There are now metabolic data available for the early life-

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J.R. Post[1] and J.A. Lee. Division of Ecology, Department of Biological Sciences, University of Calgary, Calgary, AB T2N 1N4, Canada.

[1] Author to whom all correspondence should be addressed.

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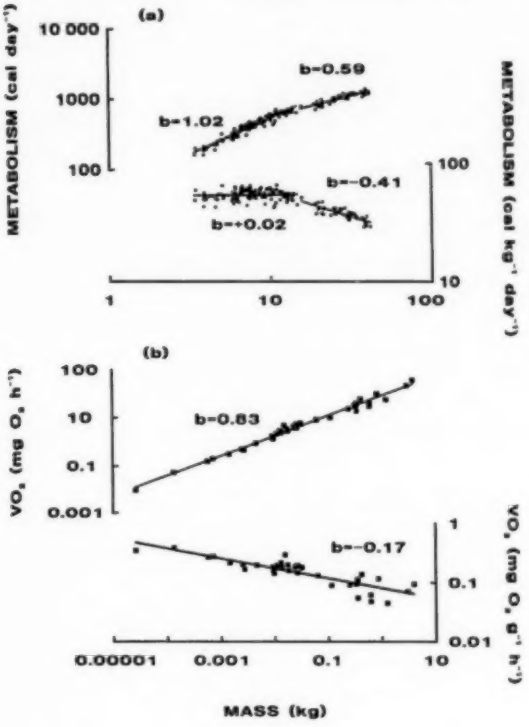
Fig. 1. Metabolic rates expressed as energy or O_2 per unit time and as mass-specific rates in energy or O_2 per unit mass per unit time for (a) human males (digitized from Brody 1945) and (b) common carp, *Cyprinus carpio* (digitized from Winberg 1956).



history stages of many species of marine and freshwater fishes. Despite the ample data, methodological differences among studies make it difficult to develop general mass scaling relationships throughout ontogeny. Reviews of metabolic rates of the early life-history stages of fish have provided partial lists of scaling regression coefficients, but the variety of metabolic units used makes it impossible to compare these results directly. These reviews also tend to focus on a few studies that identify isometric scaling, ignoring the wide variation in mass exponents that appears to be typical. Studies that purport to have identified a metabolic ontogeny similar to Brody's (1945) model lack objective statistical treatments of the data, instead eyeballing the apparent inflection and then fitting two independent curves to the data. Also, the explicit development of ontogenetic models is not possible from the majority of published studies because they examine metabolic rates only for early life-history stages, collecting no comparable data for later life-history stages of the species.

The majority of laboratory studies that measure metabolic rates of fishes measure standard or routine rates. However, an understanding of in situ metabolic rates requires measurements of the energetic costs of active metabolism. To date there have been no studies dealing with the ontogeny of active metabolism throughout the life history of teleost fish where mass varies across the complete four to seven orders of magnitude in mass.

The goals of this study are fourfold: (*i*) to examine the standard and routine metabolism–mass scaling of exogenously feeding early life-history stages of teleost fishes; (*ii*) to examine the ontogenetic pattern of standard and routine metabolism–mass scaling, identifying mass exponents and inflections to test the appropriateness of Brody's (1945) complete ontogenetic model for teleost fishes; (*iii*) to examine the ontogenetic patterns in active metabolism–mass scaling and scope for activity; and (*iv*) to identify areas of uncertainty in our understanding of ontogenetic patterns in standard and routine metabolism, active metabolism, and scope for activity, thus identifying research priorities.

The literature, units, and hypothesis tests

A large body of literature focuses on the metabolic rates of larval and juvenile fishes. Unfortunately, published compilations of that literature are insufficient to test general hypotheses about the ontogeny of metabolic rates because of inconsistencies in the units reported among studies and the selective nature of the compilations (Giguère et al. 1988; Rombough 1988*a*; Houde 1989; Kamler 1992). Comparisons among studies in which the body size of experimental fish differs are not possible because mass-specific metabolic rates are not independent of mass. Therefore, they cannot be compared across ranges in mass unless the mass exponent is empirically known (Brody 1945; Rombough 1988*a*). Often the range in mass over which metabolic rates are measured is small, producing a mass exponent that is unrepresentative of larger ranges in mass (Rombough 1988*a*; Giguère et al. 1988; Houde 1989; Kamler 1992). In addition, metabolic rates are frequently represented as a function of age, which does not allow the separation of mass-dependent and age-dependent relationships (Rombough 1988*a*).

The universality of mean relationships are sometimes accepted uncritically despite substantial variability around means. For example, in a review of 29 studies, Giguère et al. (1988) concluded that metabolic rates of larval fish scale isometrically. This conclusion appears warranted because the grand mean slope was 1.0 (specific rate slope = 0.00), but the range in slopes was 0.65–1.69 (specific rate –0.35 to 0.69), suggesting that such a general conclusion may be inappropriate.

Another limitation of current attempts to identify the ontogeny of metabolic rates is that studies typically examine metabolic rates of single life-history stages and have no comparative data on other life-history stages. Two exceptions to this observation are studies by Kamler et al. (1987) and Oikawa et al. (1991) in which metabolic rates were examined over greater than four orders of magnitude in mass. Unfortunately, in both of these studies, weak statistical analyses were applied to test for the occurrence of more than one phase in the metabolism versus mass relationship.

The approach used by both Kamler et al. (1987) and Oikawa et al. (1991) to identify changes in mass scaling relationships was somewhat subjective. It involved visually assessing the data for an inflection point in the independent variable, dividing the data set in two at that point, calculating separate linear regressions for the two data sets, and finally using ANCOVA to test the null hypothesis that the slopes from the two regressions do not differ. This approach does not objectively test the hypothesis that a data set is best fitted by

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two relationships rather than one, nor is the identification of an inflection point objective. There are four key problems that introduce subjectivity. First, it is very difficult to identify the inflection point between two scaling relationships that are plotted on log–log scales when there is variability in the data. Second, visual assessment allows no estimation of the confidence placed on the estimate of the inflection point. Third, data points at the extremes of a range in the independent variable can have undue influence on the calculated slope of the relationship, thus estimates of the slopes can be biased by nonobjective selection of the inflection point between regressions. Fourth, ANCOVA comparisons of slopes from the two apparent relationships lack the power to reject the null hypothesis because in this method, by definition, there is no overlap in values of the independent variable. It is important to test for the occurrence of an inflection point as objectively as possible in metabolism–mass scaling and to estimate its value with a statement of confidence because it is often used to infer biological mechanisms (Kamler 1972, 1992; Oikawa and Itazawa 1985, 1992*a*; Kamler et al. 1987; Oikawa et al. 1991).

Metabolic rates of fishes are reported in the literature using two basic functional forms along with a variety of transformations and units. Use of a common function and conversion to common units is necessary to facilitate comparison. The units that we have chosen to present in this review are weight-specific metabolic rates measured as O_2 consumption (Vo_2) in units of milligrams O_2 per gram per hour and mass in grams wet weight. We undertook the conversion of published values using three assumptions: (*i*) a wet to dry weight ratio of 6:1, (*ii*) an oxycalorific coefficient of 13.6 $J \cdot mg^{-1}$ O_2, and (*iii*) physical constants relating moles O_2 to grams O_2 and milligrams O_2 to millitres O_2 following standard methods (Adams and Breck 1990; Cech 1990).

Vo_2–mass relationships are reported in the literature as

$$(1) \quad Vo_2 = a(\text{MASS})^b$$

where if Vo_2 is in units of O_2 per fish per unit time, a is the intercept in the same units as Vo_2 and b is the slope. If Vo_2 is reported in units of mass-specific respiration, O_2 per gram of fish per unit time, a is also in mass-specific units. More commonly, linear regression equations are reported using a double log transformation where

$$(2) \quad \log Vo_2 = a + b \log(\text{MASS})$$

where if Vo_2 is in units of O_2 per fish per unit time, a is the intercept in units of log O_2 per fish per unit time, and b is the slope. If Vo_2 is reported in units of mass-specific respiration, O_2 per gram per unit time, then a is in log O_2 per gram per unit time and b is the slope. In this review we will report all intercepts and slopes as in eq. 1 with a as a mass-specific rate (milligrams O_2 per gram per hour). All graphic presentations of metabolic rate versus mass relationships will be on double log axes with arithmetic scales indicated.

Giguère et al. (1988) argue that Model II (also called geometric mean, functional or reduced major axis) regression is more appropriate than Model I regression for studies of mass scaling. Model II regression assumes that the measurement error in the independent and dependent variables are equal whereas Model I assumes there is measurement error in only the dependent variable (Reiss 1989). In a strict sense, the data from ontogenetic studies do not meet either of these assumptions. Both variables are measured with error but the measurement error associated with the dependent variable (metabolic rate) is typically higher than the measurement error in the independent variable (mass). Therefore, a fitting algorithm that is intermediate between models I and II in choosing the "best" slope would be most appropriate. No such algorithm exists and the vast majority of published mass scaling relationships use Model I regression (Peters 1983; Schmidt-Nielsen 1984; Reiss 1989). It should also be noted that the difference in the estimated slope between models I and II regression is typically small for mass scaling relationships because the residual sums of squares are typically small (i.e., high r^2) (Jensen 1986). In addition, the nonlinear fitting procedures we use minimize the sums of squares in only the direction of the dependent variable, and to be consistent between our results and previous results, we report only Model I regression coefficients.

The initial tabular and graphical survey of metabolic scaling relationships in larval and juvenile fishes of all species is presented in standard units as described above with no conversion for differences in temperature or methods among studies. Species-specific analysis of the ontogeny of metabolic rates involved collection of raw data either by digitizing published graphs or by the provision of raw data by the authors of the publications. Visual examination of species-specific data, compiled from several sources, suggested that the data do not follow a continuous pattern across mass. It was assumed that the differences in metabolic rates among studies could be explained by differences in experimental temperature and methodology. As a result, the data were standardized by identification of a baseline regression that predicted the highest metabolic rate for any value of mass. Each data point from all other studies was adjusted upward to that level using the following protocol:

$$\hat{Y}_{\text{base}} = aX_i^b$$

$$\hat{Y}_i = cX_i^d$$

$$Y_{\text{corr}} = (\hat{Y}_{\text{base}} - \hat{Y}_i) + Y_i$$

where $\hat{Y}_{\text{base}}$ is the predicted Vo_2 at a given mass (X_i) from the baseline relationship, a and b are the coefficients from the baseline regression, $\hat{Y}_{\text{corr}}$ is the predicted Vo_2 for a given mass (X_i) from the relationship to be corrected, c and d are the coefficients from the regression to be corrected, and Y_{corr} is the corrected Vo_2 given X_i and Y_i.

This correction procedure makes no assumptions about the magnitude of differences in experimental temperatures or differences in methodology on metabolic rates. It simply assumes that ontogenetic patterns in metabolic rates must be continuous (i.e., no step functions), without the additional assumption that the function must be smooth. The purpose of this transformation is to preserve the allometric component of the data. Consequently, the absolute value of each data point represents the metabolic rate scaled up to the highest of the experimental temperatures used, and to the highest levels of activity observed, in the studies that were used in our compilation. We will, therefore, refer to these metabolic rates as routine rather than standard rates.

The corrected data were subjected to a nonlinear, segmented fitting algorithm (Wilkinson 1990) to test the working

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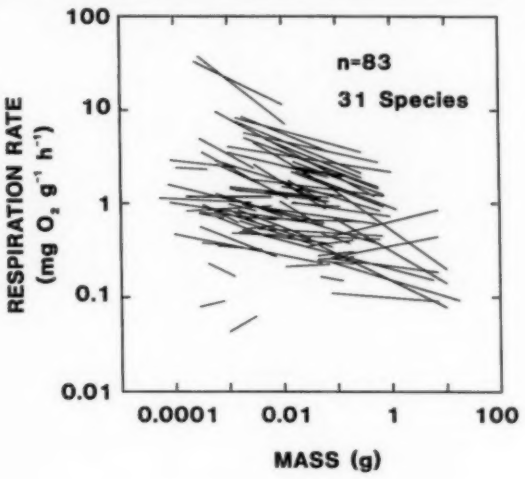
Fig. 2. Regression lines relating mass-specific respiration rates to mass from 83 studies on 31 species of teleost fishes.



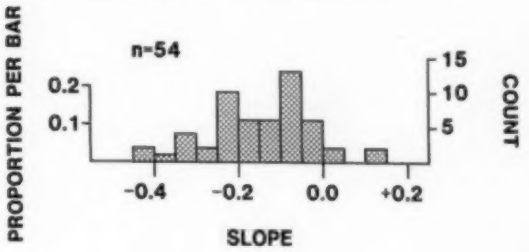
assumption that the data can be represented by two log–log linear segments each in the form of eq. 2. The robustness of the nonlinear fit was assessed in three ways. Two nonlinear fitting algorithms were used independently on the same data set and results of the hypotheses tests were compared. SYSTAT (Wilkinson 1990) uses one of two algorithms for searching for least squares minima in nonlinear estimation: (*i*) quasi-Newton, which used numeric estimates of the first and second derivatives, and (*ii*) SIMPLEX, which is an iterative direct search procedure. The second assessment of the robustness of nonlinear fitting algorithms tested the hypothesis that fitted parameters were sensitive to a small number of outliers in the data sets. To address this hypothesis, repeated nonlinear fits were done using the quasi-Newton method on random selections of 75% of the data set and the mean and standard deviation of the differences between random selections of 75% of the data and the whole data set were calculated. The third assessment involved varying initial parameter values over a large range to test the assumption that the best fit produced global rather than local solutions.

The nonlinear segmented regression model fitted to the data:

$$(3) \quad Y = B0 + B1X + B2(X - \text{BREAK})\ (X > \text{BREAK})$$

where Y is log Vo_2 (log milligrams O_2 per gram per hour), X is log mass of the fish (log grams), $B0$ is the intercept of the first segment (log milligrams O_2 per gram per hour), $B1$ is the slope of the first segment, $B2$ is the difference in slope between the first and second segments, and BREAK (log grams) is the inflection between the first and second segments. The coefficients of eq. 3 are presented in uppercase to differentiate them from the coefficients of the simple regression models more commonly used (eqs. 1 or 2). The slope $B1$ is equivalent to the slope b from eqs. 1 or 2; $B2$ is not a slope but is the difference in slope between the first and second segments. Therefore, the slope of the second segment ($b2$) is $B1 + B2$. Confidence intervals (95%) are reported for each fitted parameter.

Fig. 3. Frequency distribution of the slopes of mass-specific metabolism versus mass for only those studies reported in Fig. 2 that have a mass range of at least 10 times. There are 54 studies summarized in the frequency distribution.



The following two hypotheses were tested using this model:

H_o1: $B1 = 0$, which is equivalent to $b1 = 0$

H_o2: $B2 = 0$, which is equivalent to $b1 = b2$

Hypothesis 1 tests whether the mass exponent for early life history is isometric as presented by Brody (1945) for mammals and proposed more recently for fish (Rombough 1988*a*; Giguère et al. 1988; Oikawa et al. 1991). Hypothesis 2 tests whether mass scaling changes significantly through ontogeny, and if so, the parameter BREAK (eq. 3) is interpreted as the log mass at which scaling changes from that typical of early to later life-history phases.

Metabolic rates during early life history

A nonselective compilation of available metabolic scaling yielded 83 relationships derived from 31 species of larval and juvenile teleost fishes and identified a range of approximately 4 orders of magnitude in mass-specific respiration rates over approximately 5 orders of magnitude in wet mass (Fig. 2, Appendix 1). This broad range of specific respiration rates is probably the result of some combination of the following factors: (*i*) species-specific differences, (*ii*) differences in experimental temperature or salinity among studies, (*iii*) variation in the level of activity from standard to active by the experimental fish among studies, (*iv*) variation in the numbers of fish in experimental chambers among studies, and (*v*) variation in the level of stress exhibited by fish among studies (Brett and Groves 1979; Rombough 1988*a*; Giguère et al. 1988). If we can assume for the time being that these confounding factors act primarily on the level of metabolism rather than on how metabolism scales with mass, then we can examine the slopes of the relationships, independent of absolute metabolic rate.

Only studies in which mass range was an order of magnitude or more are included in our analysis to eliminate the bias of nonrepresentative slopes (Fig. 3). Slopes range broadly, from –0.45 to 0.14 with two modes, one near 0.00 and the other near –0.20. The range of slopes is narrower than that reported by Giguère et al. (1988) because of our slope elimination criteria but still represents a substantial range in slopes from positive to negative and does not allow rejection of either Brody's (1945) or Winberg's (1956) models.

Ontogeny of routine metabolism

Only three species of teleost fishes, common carp (*Cyprinus*

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Table 1. In vivo and in vitro respiration rate, gill area, and length allometric relationships for larval, juvenile, and adult common carp (*Cyprinus carpio*), rainbow trout (*Oncorhynchus mykiss*), and red sea bream (*Pagrus major*).

Study	Dependent variable	N	R^2	$B0$ (a_1)	$B1$ (b_1)
Common carp					
This study (compilation)	In vivo Vo_2	271	0.838	−0.501 (−0.542,−0.459)	−0.051*(−0.073,−0.030)
Kamler (1972)[a]	In vivo Vo_2	71	—	−0.380	−0.02 (−0.07,0.03)
Reanalysis of Kamler (1972)[a]	In vivo Vo_2	71	0.810	−0.422 (−0.506,−0.338)	−0.027 (−0.070,0.015)
Itazawa and Oikawa (1986)	In vivo Vo_2	25	0.999	−0.808	—
Reanalysis of Itazawa and Oikawa (1986)	In vivo Vo_2	25	0.952	−0.589 (−0.679,−0.498)	−0.093 (−0.144,−0.042)
Winberg (1956)	In vivo Vo_2	35	0.748	−0.587	—
Reanalysis of Winberg (1956)	In vivo Vo_2	35	0.757	−0.559 (−0.755,−0.363)	−0.087 (−0.303,0.128)
Itazawa and Oikawa (1986)	In vitro Vo_2	14	0.938	−0.984	—
Oikawa and Itazawa (1985)	Gill area	20	0.99	3.125	0.222
Reanalysis of Oikawa and Itazawa (1985)	Gill area	20	0.840	3.089 (2.888,3.291)	0.195* (0.081,0.309)
Oikawa and Itazawa (1985)	Length	28	0.999	−1.405	−0.792
Reanalysis of Oikawa and Itazawa (1985)	Length	28	0.999	−1.606 (−1.626,−1.586)	−0.794* (−0.803,−0.785)
Rainbow trout					
This study (compilation), fit 1[c]	In vivo Vo_2	93	0.806	−0.410 (−0.430,−0.390)	−0.026*(−0.045,−0.008)
This study (compilation), fit 2[d]	In vivo Vo_2	93	0.779	−0.334 (−0.394,0.294)	0.020 (−0.018,0.058)
Hughes 1989	Gill area	—	—	—	2.443
This study (compilation), routine[e]	In vivo Vo_2	36	0.992	−0.990 (−1.045,−0.934)	−0.049 (−0.136,0.038)
This study (compilation), active[e]	In vivo Vo_2	35	0.954	−0.471 (−0.531,−0.411)	0.095* (0.001,0.189)
Sea bream					
Oikawa et al. (1991)	In vivo Vo_2	39	0.997	0.031	−0.051
Reanalysis of Oikawa et al. (1991)[f]	In vivo VO_2	35	0.977	−0.157 (−0.329,−0.015)	−0.014 (−0.072,0.044)
Oikawa and Itazawa (1992)	In vitro Vo_2	48	0.990	−0.346	−0.085
Reanalysis of Oikawa and Itazawa (1992)	In vitro Vo_2	47	0.953	−0.712 (−2.272,0.849)	−0.095 (−0.561,0.370)
Reanalysis of Oikawa et al. (1991)[f]	length	99	0.999	1.470 (1.444,1.496)	−0.746* (−0.756,−0.736)

Note: Simple linear and segmented nonlinear regressions are reported from the literature, our reanalysis of the literature data, and analysis of compilations of literature data. See the text for the derivation and explanation of the regression coefficients. Confidence limits (95%) are indicated in parentheses for all fitted parameters. Rejection of the null hypothesis for coefficients $B1$ and $B2$ is indicated by an asterisk. A significant $B1$ indicates that the slope of the first segment of the segmented regression is significantly different from zero. A significant $B2$ indicates that the difference between the slope of the first segment and the second segment is nonzero, indicating that the two slopes are significantly different. Note that if $B2$ is not significant (i.e., the confidence intervals include zero) then the fitted inflection point, BREAK, is not significant. The data compiled from various authors for analysis in this study were digitized from the original publications or kindly provided by the authors. Data compiled for common carp were from Winberg (1956), Beamish (1964), Kamler (1972), Kamler et al. (1987), Oikawa and Itazawa (1984), and Itazawa and Oikawa (1986). Data compiled for rainbow trout were from Rao (1968), Staples and Nomura (1976), Rombough (1988*a*), and Wieser et al. (1985).

[a]Raw data kindly provided by E. Kamler (Institute of Ecology, Polish Academy of Sciences, Dziekanow Lesny, 05–092 Lomianki, Poland).

[b]Estimated by eye from the raw data by original author.

[c]Coefficient from fit 1 with low local minima in the inflexion coefficient.

[d]Coefficient from fit 2 with high local minima in the inflection coefficient.

[e]Coefficients for routine and maximally active metabolic rates for rainbow trout derived from data digitized from Rao (1968) at 4°C and Wieser (1985) at 5°C.

[f]Raw data kindly provided by S. Oikawa (Department of Fisheries, Faculty of Agriculture, Kyushu University 46–04, Higashi-ku, Fukuoka 812, Japan).

carpio), rainbow trout (*Oncorhynchus mykiss*), and red sea bream (*Pagrus major*), provide sufficient data to examine metabolism throughout complete life histories. As explained earlier, the data for each species were corrected to produce a smooth relationship between metabolic rate and mass with no implicit assumptions about the effect of temperature or levels of metabolism (i.e., standard or routine) among studies.

The largest data set is for common carp with 271 data points that cover 6 or 7 orders of magnitude in mass (Fig. 4). The nonlinear fitting procedure identified two segments with significantly different slopes. There was a significant negative, but shallow, slope for the first phase (−0.051) and a significantly steeper negative slope for the second phase (−0.179) (Table 1). The fitted inflection point that separates phase 1 from phase 2 is 0.290 g, which is substantially larger than size at metamorphosis for the common carp. Kamler (1972, 1992) and Kamler et al. (1987) visually assessed the inflection in mass scaling in their data set to be 1.0 g for common carp. Our analysis of their data using a segmented regression (eq. 3) yielded an inflection point of 0.240 g with 95% confidence intervals of 0.195–0.294 g (Table 1). This biphasic relationship for exogenously feeding common carp is in agreement with a smaller data set from Kamler (1972, 1992) and Kamler et al. (1987), but it conflicts with data sets from Winberg (1956) and Itazawa and Oikawa (1986).

Reanalysis of the data sets of Winberg (1956) and Itazawa and Oikawa (1986) using eq. 3 showed that the slopes of the first phase were close to zero and that those of the second phase were substantially more negative but in neither case were the phase 1 and phase 2 slopes significantly different from one another (Table 1). In both cases, the sample size was small (n = 35, 25) rendering the asymptotic standard errors

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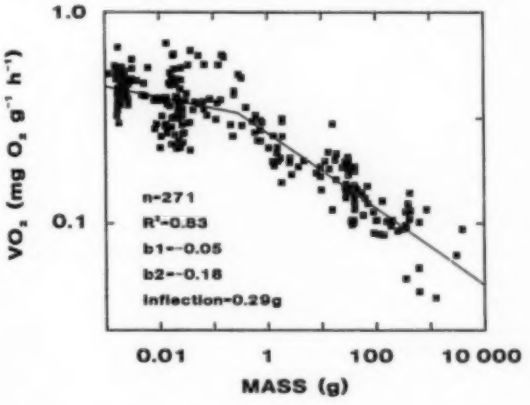
$B2$ (b_2–b_1)	b2 ($B1$+$B2$)	BREAK	Inflection (g)
–0.128*(–0.162, –0.094)	–0.179	–0.538 (–0.597, –0.478)	0.290 (0.253,0.333)
—	–0.20	—	1.0[b]
–0.180*(–0.248,–0.112)	–0.207	–0.620 (–0.709,–0.531)	0.240 (0.195,0.294)
—	–0.168	—	—
–0.044 (–0.107,0.019)	–0.137	–1.178 (–1.328, –1.027)	0.066 (0.047,0.094)
—	–0.168	—	—
–0.095 (–0.316,0.126)	–0.182	0.008 (–2.302,2.317)	1.019 (0.005,207)
—	–0.153	—	—
—	–0.206	—	0.33[b]
–0.403* (–0.534,–0.272)	–0.208	–0.366 (–0.933,0.201)	0.431 (0.117,1.589)
—	–0.664	—	0.150–0.357[b]
0.135* (0.125,0.145)	–0.659	–0.840 (–0.957,–0.723)	0.145 (0.110,0.192)
–0.213*(–0.259,–0.166)	–0.239	0.749 (0.694,0.803)	5.61 (4.94,6.35)
–0.180*(–0.227,–0.134)	–0.160	–0.274 (–0.595,0.047)	0.532 (0.254,1.11)
—	–0.068	—	0.1[b]
–0.155*(–0.310,–0.001)	–0.204	0.811 (–0.272,1.893)	6.47 (0.530,78.2)
–0.215* (–0.380,–0.051)	–0.120	0.648 (0.592,0.705)	4.45 (3.91,5.07)
—	–0.179	—	0.004–0.007[b]
–0.162*(–0.226,–0.097)	–0.176	–2.611 (–2.670,–2.552)	0.002 (0.002,0.003)
—	–0.235	—	0.003–0.005[b]
–0.167 (–0.633,0.299)	–0.262	–3.019 (–4.063,–1.976)	0.001 (0.00009,0.011)
0.068* (0.057,0.079)	–0.678	–2.225 (–2.265,–2.186)	0.006 (0.005,0.007)

large (2–6 times higher than for the complete data set with $n = 271$), and therefore, the 95% confidence intervals are wide. Thus, there is poor power to reject the null hypothesis of no difference. For the two largest data sets, our compilation and combined data from Kamler (1972) and Kamler et al. (1987), the null hypothesis that the difference between the slopes of phase 1 and phase 2 was zero was rejected, indicating that phase 2 had a significantly more negative slope than did phase 1 (Table 1).

The second largest data set is for rainbow trout with 93 data points that cover 6 or 7 orders of magnitude in mass (Table 1, Fig. 5). The nonlinear fitting procedure identified two segments with significantly different slopes. The first was nearly isometric and the second, allometric (Table 1). The nonlinear fitting procedure identified two inflections for the change from $b1$ to $b2$, probably due to the low data density in the independent variable in the region of the uncertainty. At the higher breakpoint (5.6 g; Table 1, fit 1), $b1$ (–0.026) is significantly different from 0 and significantly different from $b2$ (–0.239; Table 1, fit 2). At the lower breakpoint (0.53 g; Table 1, fit 2), which had a marginally poorer fit (fit 1, $R^2 = 0.806$; fit 2, $R^2 = 0.779$), $b1$ (–0.020) was not significantly different from 0 but was significantly different from $b2$ (–0.16). Rombough (1988*b*) demonstrated that rainbow trout had isometric scaling from fertilization to the time of complete yolk absorption at approximately 0.2 g wet weight. Both of our fitted inflection points are larger than rainbow trout size at complete yolk absorption.

The smallest data set relating metabolic rates to mass over a range of 6 or 7 orders in magnitude in mass is for sea bream and consists of 39 data points (Table 1, Fig. 6). The postembryonic stages of sea bream exhibit two mass exponents; $b1$ is not significantly different from zero (–0.071) but is significantly

Fig. 4. Mass-specific respiration rate versus mass data for common carp, *Cyprinus carpio*, from six studies. The segmented regression, sample size, R^2, the slopes of the first ($b1$) and second ($b2$) segment, and the inflection point are shown. The data were extracted from Winberg (1956), Beamish (1964), Kamler (1972), Kamler et al. (1987), Oikawa and Itazawa (1984), and Itazawa and Oikawa (1986).



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different than $b2$ (–0.176). The inflection point of 0.0024 g is substantially lower than mass at metamorphosis, which occurs at approximately 10 mm in length (Fuiman and Webb 1988) corresponding to a mass of approximately 0.01 g (Oikawa et al. 1991).

All three species for which there was sufficient metabolic rate data from postembryonic stages showed a biphasic ontogeny qualitatively similar to that shown for postembryonic mammals by Brody (1945). Mass scaling for the first phase of all three species had a negative slope, with an exponent close to zero but that was significantly less than zero, for two of the three species. This suggests that Giguère's et al. (1988) conclusions that larval fish have isometric metabolism–mass scaling may not be generally applicable. Mass scaling for the second phase in all three species had allometric mass exponents, significantly more negative than phase 1 coefficients, which were similar to the general mass scaling exponents ($b = -0.20$) suggested by Winberg (1956) as appropriate for fish. The mass that marked the inflection in scaling from phase 1 to phase 2 differed among species by greater than two orders of magnitude. These inflections do not correlate with size at metamorphosis in any of the species, and the point of inflection was larger than size at metamorphosis in two species and smaller than size at metamorphosis in one species.

The robustness of the nonlinear fitting procedure was tested for sensitivity to (*i*) initial values, (*ii*) solution methods, and (*iii*) outliers. The fitted parameters were not sensitive to initial values for common carp or sea bream but were sensitive to the parameter BREAK for the rainbow trout fit. In rainbow trout, two local minima were found for BREAK, one with a low and one with a high initial value. Visual examination of the rainbow trout data identified a low density of data in the region of the two local minima, so this parameter must be viewed cautiously. The two solution methods used in the SYSTAT NONLIN procedure gave identical results for the hypothesis tests for the common carp and rainbow trout data sets, which had large degrees of freedom (df = 270, 92), but different results for sea bream (df = 33). The disagreement between solution methods for sea bream is probably due to the high asymptotic errors, which were approximately an order of magnitude higher for sea bream than for either common carp or rainbow trout. This reduced the power to reject the null hypothesis.

Sensitivity of parameter estimates and hypothesis tests to outliers was assessed by doing 30 nonlinear fits on random permutations of 75% of the data sets. The coefficient of variation of parameter estimates ranged from 4 to 16% for these subsets of the complete data and in all cases the results of the hypothesis tests were identical to the results from the complete data sets. We therefore conclude that the nonlinear fitting procedure is robust but caution that small sample size or variation in data density across the range of values for the independent variable can compromise the power of the analyses. Therefore, we must cautiously interpret our test of hypothesis 1 for sea bream and our estimate of the parameter BREAK for rainbow trout. Thus, we have reported both fits for rainbow trout and the single best fit for common carp and sea bream from the quasi-Newton method (Wilkinson 1990).

There have been three general hypotheses advanced to explain the biphasic ontogenetic pattern in metabolism–mass scaling observed in some species: (*i*) changes in the relative sizes of respiratory surfaces during ontogeny, (*ii*) changes in swimming efficiency through ontogeny, and (*iii*) differential recruitment rates of organs and tissues that vary in their level of metabolic activity. The type of data that has been accumulated to date is only appropriate to provide correlative evidence for these hypotheses rather than experimental tests of the occurrence of particular physiological processes. We now examine the strengths and weaknesses of this data in support of the explanatory hypotheses.

It is clear that teleost fishes undergo a rapid expansion in gill surface area after hatch. The increase in gill surface area is associated with an increase in metabolism during a period when there is no change in mass (DeSilva 1974; Oikawa and Itazawa 1985; Hughes and Al-Kadhomiy 1988; Hughes 1989; Rombough and Moroz 1990). The hypothesis that changes in the relative proportion of gill area and cutaneous area through ontogeny may be correlated with a biphasic metabolism–mass allometry for exogenously feeding fishes has been proposed (Dabrowski 1986*a*; Hughes and Al-Kadhomiy 1988; Rombough and Moroz 1990; Kamler 1992). The proposed mechanism is simply a surface area – volume argument. Ontogenetic changes in volume (mass) would be expected to incrementally limit O_2 transfer as surface area – volume ratios decreased.

A biphasic metabolism–mass ontogeny would result if the following three processes occurred: (*i*) gill surface area increased in proportion to volume during early development, (*ii*) total surface area decreased in proportion to volume throughout development, and (*iii*) respiratory surfaces are limiting to routine metabolic rates throughout development. It is generally accepted that the third mechanism sets the upper limit to the rate of maximum aerobic metabolism (Oikawa and Itazawa 1985; El-Fiky and Wieser 1988; Kaufman 1990), but it might also be true that it is involved in delimiting standard and routine metabolism through allometric effects on oxygen transfer efficiency.

Oikawa and Itazawa (1985) demonstrated biphasic gill area – mass and surface area – mass allometries for common carp. They compared these allometries with Winberg's (1956) single slope regression for metabolic rate versus mass and concluded that "gill, body surface and fin areas do not directly regulate the resting metabolism of fish." In contrast to Winberg's (1956) analysis, our compilation of carp $\dot{V}O_2$ and mass data show a biphasic allometry of metabolic rates and substantial congruence between the $\dot{V}O_2$–mass, gill area – mass, and length–mass inflection point estimates in the range of 0.1–0.5 g (Table 1). Oikawa et al. (1991) estimate slopes and inflection in $\dot{V}O_2$–mass and length–mass relationships for sea bream but do not interpret the results. Our reanalysis of their data identifies similar inflection points for the two relationships (Table 1). Our compilation and analysis of rainbow trout data also identify, although imprecisely, an inflection in $\dot{V}O_2$–mass allometry similar to the inflection eyeballed by Hughes (1989) for gill area – mass data. We, therefore, conclude that ontogenetic patterns in the relative contribution of various respiratory surfaces can explain ontogenetic patterns in $\dot{V}O_2$–mass scaling. The sustained, high levels of $\dot{V}O_2$ at pre-inflection masses can be explained on the basis of expansion in the gill surface area relative to mass. A reduction in specific $\dot{V}O_2$ rates at post-inflection masses may also be constrained by a reduction in the gill area relative to mass.

Comparisons between in vivo and in vitro metabolic allometries are useful to test the respiratory surface area – mass

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Fig. 5. Mass-specific respiration rate versus mass data for rainbow trout, *Oncorhynchus mykiss*, from four studies. The segmented regression, sample size, R^2, the slopes of the first ($b1$) and second ($b2$) segment, and the inflection point are shown. Two nonlinear fits are indicated, one with a solid line, the other with a broken line corresponding to the first and second set of parameter estimates (see text for an explanation). The data were extracted from Rao (1968), Staples and Nomura (1976), Rombough (1988*a*), and Wieser et al. (1985).

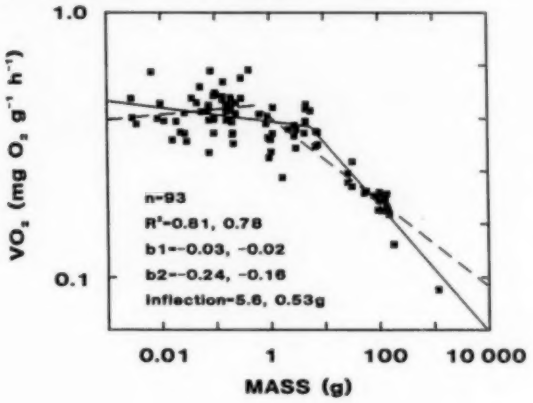
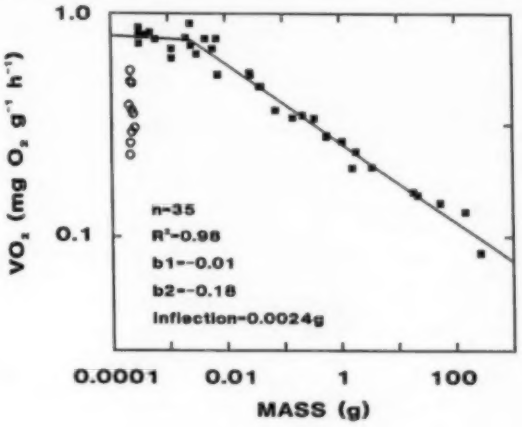


Fig. 6. Mass-specific respiration rate versus mass data for sea bream, *Pagrus major*, from one study. The segmented regression, sample size, R^2, the slopes of the first ($b1$) and second ($b2$) segment, and the inflection point are shown. The open circles are for endogenously feeding embryos and are not included in the segmented regression. The data were extracted from Oikawa et al. (1991).



hypothesis. Congruent in vivo and in vitro metabolic allometries would suggest that surface area to volume constraints to oxygen transfer are not operating because in vitro respiration is unimpeded by rates of oxygen transfer. Our compilation of in vivo metabolic rates for common carp identified a significant inflection in metabolic rates as a function of mass. An inflection was not found in vitro (Itazawa and Oikawa 1986), although the power was low because of small sample size. If Itazawa and Oikawa's assessment of a uniphasic in vitro metabolic allometry is correct, the lack of congruence between in vivo and in vitro allometries supports the surface area – volume hypothesis.

A biphasic ontogeny in common carp shape (Oikawa and Itazawa 1985; and our reanalysis of their data) identified a change from higher to lower surface area to volume ratios (length to weight) from the first to second phase with an inflection point similar to that for the Vo_2–mass relationship. This observation is also consistent with the hypothesis that ontogenetic changes in respiratory surface to mass ratios control Vo_2–mass relationships throughout ontogeny.

The second major hypothesis that may explain a biphasic Vo_2–mass ontogeny involves changes in swimming efficiency through life history. There are two components to swimming efficiency: (*i*) the type, quantity, and O_2 consumption of the muscles responsible for swimming and (*ii*) the hydrodynamic constraints imposed by the environment. Swimming muscles are not fully differentiated until many weeks after hatching, and the proportion of muscle mass to total mass as well as the ratio of red to white muscle fibres increase throughout larval development. All of these changes should improve swimming efficiency (Forstner et al 1983; Wieser et al. 1985; Dabrowski 1986*b*).

In addition to physiological changes in the fish during early development, there are dramatic changes in the hydrodynamic regime in which the fish must perform. The development from newly hatched larvae through the period of exogenously feeding larvae is associated with a change from viscous to laminar hydrodynamics, which dramatically affects the efficiency of acceleration (Fuiman and Webb 1988). This shift in hydrodynamic environment is often correlated with a shift from anguilliform to subcarangiform or carangiform swimming styles (Hunter 1972; Batty 1981, 1984; Dabrowski 1986*b*) and a shift in foraging ability (Blaxter 1969; El-Fiky and Wieser 1988). However, it is not clear from the literature whether these swimming efficiency mechanisms will result in a distinct threshold change in swimming efficiency and result in a biphasic metabolism–mass relationship.

The third process that has been proposed to explain the biphasic Vo_2–mass relationship for exogenously feeding fish is the timing of recruitment of organs and tissues that differ in metabolic demand throughout ontogeny. Oikawa et al. (1991) propose that the isometric Vo_2–mass relationship during the post-larval stage "is considered to be caused by more rapid growth of organs with high metabolic activity such as brain and alimentary canal." This conclusion was based on earlier work by Itazawa and Oikawa (1986) that shows that organs of high metabolic activity, such as the brain and kidney, get smaller relative to tissues and organs of lower metabolic activity, such as trunk and white muscle, as the fish increases in size. Unfortunately, only slopes were presented for these tissue-specific Vo_2–mass relationships. Without estimates of the goodness of fit to a linear hypothesis we are not able to assess whether this mechanism can explain the biphasic in vivo Vo_2–mass relationships in the species. Oikawa et al. (1991) and Oikawa and Itazawa (1992*a*) attempt to strengthen their proposal (that the observed biphasic Vo_2–mass allometry is caused by changes in the relative proportion of tissues and organs that differ in metabolic activity) by rejecting the alternate respiratory surface area – volume hypothesis. They contrast

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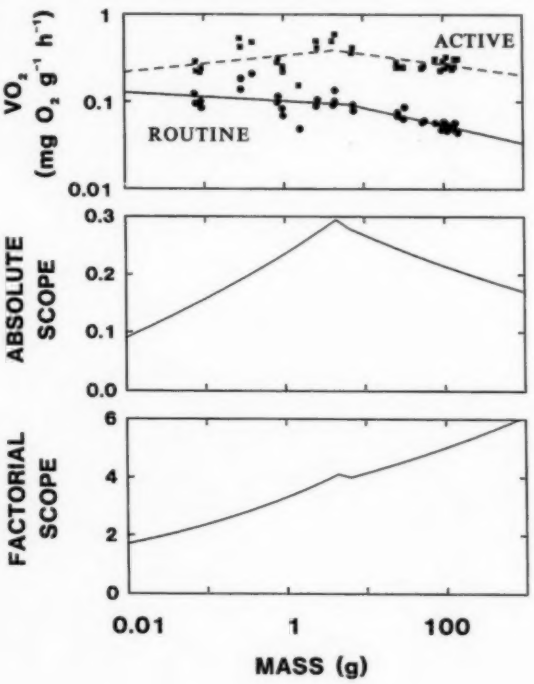
$\dot{V}O_2$–mass relationships in vivo with in vitro for sea bream and purport to identify biphasic allometry with similar inflection points. Because in vitro tissues are not subjected to surface area – volume constraints they reject this explanation as the mechanism producing a biphasic in vivo $\dot{V}O_2$–mass ontogeny. Unfortunately, Oikawa et al. (1991) and Oikawa and Itazawa (1992*a*) did not use an objective criterion to fit the position of the inflection and, therefore, the slopes of the two phases. Our reanalysis of the in vitro data showed that the slopes of the first and second phases do not differ, and therefore, there is no inflection point in the relationship (Table 1). As a result we cannot reject the surface area limitation hypothesis in favour of the differential tissue and organ recruitment hypothesis as did Oikawa et al. (1991) and Oikawa and Itazawa (1992*a*).

What can we say about the processes involved during ontogeny that may explain a biphasic $\dot{V}O_2$–mass relationship given reevaluation of data? Hypotheses involving constraints to respiratory rates based on surface area to volume considerations are viable and are supported by the congruence of inflection points in $\dot{V}O_2$–mass, gill area – mass, and length–mass relationships (Table 1). Clearly, there are ontogenetic patterns in muscle mass, physiology, and hydrodynamic regime that, in aggregate, could produce a biphasic pattern in swimming efficiency (and, therefore, $\dot{V}O_2$), but there are no studies that address these processes throughout ontogeny of any single species. The differential tissue and organ recruitment rate hypothesis has not been strongly supported by data, but if it could be demonstrated that, in the aggregate, tissues and organs varying in metabolic activity produce a biphasic $\dot{V}O_2$–mass relationship, this would provide strong support for this hypothesis. Therefore, the functional hypothesis for which there is the strongest correlational evidence is the surface area – volume hypothesis. However, one must bear in mind that there are currently no strong data available that allow rejection of the alternate hypotheses. An explanation of the observed correlations requires a better understanding of the processes that create ontogenetic changes in energy demand in fishes.

Fig. 7. Routine mass-specific respiration rates (solid line and circles) and maximal aerobic active respiration rates (broken line and squares) as a function of mass for rainbow trout. The juvenile rainbow trout relationships are from Wieser (1985) at a temperature of 4°C and the adult relationship from Rao (1968) at 5°C. The lines are fitted with a segmented regression model and coefficients are reported in Table 1. Absolute and factorial scopes for activity are calculated from the fitted mass scaling for routine and active respiration rates.



Ontogeny of active metabolism

To understand the ontogenetic patterns in the limits to in situ metabolic rates we need to understand the relationships between routine metabolic rates and aerobic metabolic rates associated with maximal rates of activity. Data on routine and maximal aerobic metabolic rates that cover large components of the life history were found in the literature for only one species of teleost fish, rainbow trout (Rao 1968; Wieser 1985).

Early life-history mass scaling of routine metabolic rates of rainbow trout has a slope not significantly different from zero. A significantly more negative mass scaling results for later life-history stages with a slope near –0.2 (Table 1, Fig. 7). The mass scaling of routine metabolic rates was significantly different from that of active metabolic rates during early life history and was more negative for routine than active metabolism during later life history. The inflection points for routine and active metabolism mass scaling did not differ significantly (Table 1, Fig. 7).

Absolute scope for aerobic activity in rainbow trout (maximal aerobic metabolic rate - routine metabolic rate) increases through ontogeny to the point of inflection in $\dot{V}O_2$ mass scaling and then declines to approximately double the absolute scope observed for early life-history stages (Fig. 7). Factorial scope for activity (maximal aerobic metabolic rate / routine metabolic rate) increases from approximately 2 to 6 throughout ontogeny (Fig. 7). An increase in factorial scope for activity with mass has also been observed elsewhere in studies of salmonids, although the observations were made across narrower ranges in mass (see reviews by Brett and Groves 1979; Rombough 1988*a*; Kamler 1992). Mass independence of factorial scope for activity has also been observed (*Rutilis rutilis*, *Leuciscus cephalus*, *Scardinius erythrophthalmus*; Wieser and Forstner 1986). The highest observed scopes for activity that have been observed are for salmonids that undergo, or at least are capable of, long-range migrations at high sustained swimming speeds.

A more detailed assessment of ontogenetic patterns in factorial scope for activity from published data is difficult because of two potential problems with extant measurements (Rombough 1988*a*). First, standard metabolism is theoretically the baseline to which active metabolism is compared to estimate scope. Because most studies typically measure rates at some unspecified level above standard metabolism, standardization among studies is difficult (Rombough 1988*a*). For example, in our compilation of metabolic allometry for rainbow trout, our baseline metabolic measurements are more

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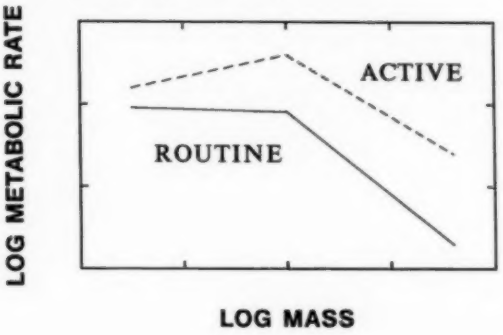
Fig. 8. A general ontogenetic pattern in mass specific routine (solid line) and active (broken line) metabolic rates as a function of mass in teleost fishes.



characteristic of routine than standard rates (see discussion of the transformation used to preserve allometry). Therefore, the factorial scope for activity that we present is lower than would be obtained if we had a true measure of standard metabolism. Second, it is difficult to measure active respiration in a repeatable way across the whole life history because the mode of activity often varies with developmental stage (Rombough 1988*a*). In early life-history stages, activity is typically of short duration (i.e., bursts of activity followed by relative quiescence) and in older fish is more often of longer duration (i.e., sustained swimming) (Wieser 1985; Wieser et al 1985; Rombough 1988*a*; Kaufman 1990; Kamler 1992).

Given these caveats and recognizing that there are no species for which a carefully controlled series of standard and maximally active metabolic rates have been measured consistently throughout ontogeny, we propose that the ontogenetic pattern in scope for activity will vary among species that differ in their life-history strategies. There are also obvious constraints on the maximum scope for activity that are independent of life-history strategy. The costs of maintenance must be paid through standard metabolism and there is an upper limit to maximum aerobic metabolism, which is constrained by the maximum rate that oxygen can be transported to oxidizing tissues (Kaufman 1990). These constraints apply throughout life history, and in addition, in the earliest life-history stages when fish grow at high rates there is an additional constraint on scope for activity. High growth rates require high rates of O_2 consumption, and therefore, growth competes with activity for O_2 supply (Wieser et al. 1988; Wieser and Medgyesy 1990*a*). The implication is that there is a dynamic balance between maintenance metabolism, activity metabolism, and growth metabolism in larval fish when faced with the necessities to (*i*) grow quickly to avoid mortality from a size-structured, gape-limited predator community, (*ii*) forage actively to maximize intake rates of patchily distributed prey, and (*iii*) maintain physiological and biochemical processes and condition. The processes involved in this dynamic balance are reasonably well known, but the solution to the tradeoffs among growth, activity, and maintenance is poorly understood and would be expected to vary with life-history stage. For these reasons, tradeoffs certainly warrant further research from both the proximate and evolutionary perspectives.

Proposed general metabolic ontogeny

We propose a general ontogenetic model of routine metabolism and scope for activity for teleost fishes generated from this review and analysis of published data (Fig. 8). The routine metabolism model is qualitatively similar to the biphasic scheme proposed for mammals by Brody (1945). In the first phase, a period of rapid growth, which often covers one to four orders of magnitude in mass, metabolism–mass scaling coefficients are negative but close to the isometric scaling reported for mammals (Brody 1945). In the second phase of ontogeny, covering large juveniles and adults, metabolism–mass scaling is clearly allometric with coefficients close to –0.20, which was proposed for fish by Winberg (1956). This general model is supported by our analysis of the data for the common carp, rainbow trout, and sea bream across the full range in mass over the full life history.

A survey of metabolism–mass scaling for early life-history stages of 31 species of teleosts demonstrated that there is substantial variability in the mass exponents during the fast growth phase. It is unclear whether this variability in mass exponents indicates that our proposed biphasic model can not be generalized or merely that mass exponents differ among species.

The position of the inflection in metabolic scaling varied dramatically among the species for which there were sufficient data to fit the model. The inflection point appears not to be correlated to size at metamorphosis. Additional experimental research and in situ observations will be necessary to untangle the ecological and evolutionary processes responsible for the observed shift in metabolic scaling from near isometric to strongly allometric at some intermediate mass. The functional explanation for the occurrence and position of the inflection is unclear, but it is correlated to ontogenetic changes in the mass scaling of respiratory surfaces. A high, and approximately constant, respiration rate during the isometric (or near isometric) stage could be attributed to a combination of efficient O_2 transfer across cutaneous surfaces and the rapid development of gill lamellar surface area. This implies that gills are fully formed and functional at the inflection point, and the efficiency declines exponentially as the surface area to volume ratio decreases during this phase of rapid growth in volume (mass). This explanation should be considered somewhat speculative because there are not sufficient empirical data to reject the two alternate hypotheses of ontogenetic changes in swimming efficiency or the differential recruitment of organs and tissues that vary in metabolic demand.

A series of experiments could be designed to differentiate among these mechanisms, but they will have to overcome substantial technical difficulties. Experiments must standardize methods over all life-history stages from embryo to large adults. Measures of respiration will have to be partitioned into gill and cutaneous respiration. Currently, published results assess only changes in respiratory surface area, and this measure does not estimate respiratory intensity because gas exchange efficiency is tissue specific. Partitioning respiratory intensity into gill and cutaneous sources is technically feasible for large fish (Cech 1990), but small body sizes pose limitations. Finding congruent mass scaling inflection points with both total and proportional respiration would provide strong support for the respiratory hypothesis. The swimming efficiency hypothesis could be tested by simultaneously measuring

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the relative proportion of muscle fibre types, hydrodynamic regime, and activity-dependent respiration rates through ontogeny. The differential recruitment rate hypothesis could be tested using a protocol similar to that of Itazawa and Oikawa (1986). Experiments should include measurement and analysis of the mass scaling of each tissue and organ independently, the mass scaling of the aggregate of all tissues and organs, and the mass scaling of the whole organism through ontogeny. Differential scaling of various tissues and organs that, in aggregate, have biphasic scaling with the same inflection as that determined in vivo would be strong supporting evidence for this hypothesis.

Although uncertainties remain, a pattern of routine metabolism during ontogeny is emerging and it is much clearer than the ontogenetic pattern of activity metabolism and scope for activity. One of the reasons for this uncertainty is that only one species of teleost fish has sufficient data throughout its life history to assess ontogeny of scope for activity. There appear to be upper limits to scope for activity that are best characterized for salmonids for which factorial scopes of 10–12 appear to be maximal (Brett and Groves 1979; Rombough 1988*a*). Aerobic activity metabolism is probably constrained at the maximum by the ability of the respiratory system to supply O_2 at all life-history stages and in particular for early life stages constrained by the tradeoffs involving the metabolic demands of growth, activity, and maintenance. Once established, ontogenetic patterns in scope for activity would be very useful as they delimit maximum aerobic activity and, therefore, constrain behaviour of fish in situ. Clearly, more research is necessary to establish ontogenetic patterns in scope for activity and, more importantly from an ecological perspective, to establish the realized rather than just the minimum (standard) and maximum (scope for activity) in situ metabolic costs.

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Appendix 1. Metabolic allometry for 31 species of larval and juvenile teleost fishes.

Species	Temp (°C)	Mass range		Allometric coefficients		Activity level	Source[a]
		Minimum	Maximum	*a*	*b*		
Abramis ballerus	20	0.0156	0.912	0.726	–0.08	R	1
Abramis brama	20	0.0016	0.0403	0.202	–0.18	R	2
Achirus lineatus	28	0.0000858	0.00149	1.67	–0.0584	R	3
Alburnus alburnus	20	0.0018	0.516	0.579	–0.08	R	1
	20	0.0006	0.48	0.960	–0.31	R	4
Anabas testudineus	28	0.005	0.05	1	–0.05	R	5
Anchoa mitchilli	26	0.0000534	0.00255	0.899	–0.023	R	3
Archosargus rhomboidalis	26	0.000109	0.000397	2.07	–0.0162	R	3
Chalcalburnus chalcoides	20	0.0018	0.065	1.33	–0.14	R	6
	20	0.0018	0.065	1.97	–0.23	AF	7
	20	0.002	0.008	0.726	0.048	S	7
	20	0.008	0.57	1.28	–0.224	S	7
	20	0.002	0.57	1.34	–0.213	R	7
	20	0.002	0.57	2.60	–0.126	AS	7
Channa puctatus	28	0.001	1.00	2.2	–0.088	R	8
Clupea harengus	10	0.0006	0.6	0.765	–0.177	R	9
	10	0.0006	0.6	0.338	–0.177	AN	9
	8	0.00103	0.00306	0.138	–0.263	R	10
	13	0.00103	0.00306	0.424	0.331	R	10
	18	0.00103	0.00306	0.389	–0.134	R	10
Coregonus schinizi	14.5	0.053	0.167	0.2723	–0.184	R	11
Coregonus wartmanni	15	0.01	0.12	0.987	–0.091	S	12
	15	0.01	0.12	1.582	–0.099	AF	12
Cyprinus carpio	20	0.00116	0.6	0.416	–0.02	R	13
	20	0.0036	0.674	0.448	–0.03	R	14
	26	0.002	0.250	0.632	–0.016	R	15
Gadus morhua	4	0.000339	0.00146	0.0940	–0.291	R	16
	7	0.000300	0.00730	0.0899	–0.225	R	16
	10	0.000330	0.0121	0.286	–0.313	R	16
Hypophthalmicthys molitrix	20	0.0001	0.05	0.168	–0.11	R	17
	20	0.0700	17.2	0.152	–0.17	R	17
Heteropneustes fossilis	28	0.0018	5.7	0.2	–0.15	R	18
Leistomus xanthurus	10	0.0109	0.0481	0.424	–0.3	R	19
	15	0.0131	0.0616	0.368	–0.36	R	19
	20	0.000084	0.0535	0.475	–0.08	R	19
Limanda limanda	10	0.09	10	0.308	–0.334	R	20
Melanogrammus aeglefinus	4	0.000419	0.00124	0.0282	–0.267	R	16
	7	0.000302	0.00290	0.380	–0.316	R	16
	9	0.000286	0.0112	0.953	–0.450	R	16
Micropogonias undulatus	10	0.009	0.0386	0.210	–0.34	R	19
	15	0.0114	0.0703	0.233	0.02	R	19
	20	0.000078	0.0559	0.419	–0.14	R	19
Micropterus dolomieui	20	0.00265	0.00975	0.274	–0.21	R	21
Morone saxatilis	18	0.00024	0.0100	3.22	–0.279	R	22
Oncorhynchus mykiss	4	0.080	7.00	0.0992	–0.04	S	23
	4	0.080	7.00	0.352	0.11	AS	23
	12	0.080	7.00	0.214	–0.07	S	23
	12	0.080	7.00	0.637	0.14	AS	23
	6	0.045	0.196	0.218	–0.0314	R	24
	9	0.045	0.196	0.281	0.0328	R	24
	12	0.045	0.196	0.331	0.0546	R	24
	15	0.030	0.193	0.447	0.0677	R	24
Pagrus major	20	0.00031	0.0073	0.525	–0.051	R	25
Perca fluviatilis	20	0.0015	0.0317	0.202	–0.22	R	2
Pleuronectes platessa	10	0.05	10	0.150	–0.28	R	20
	10	0.05	10	0.516	–0.41	A	20
_____	10	0.003	0.06	0.620	–0.34	R	9

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Appendix 1 (*concluded*).

Species	Temp (°C)	Mass range		Allometric coefficients		Activity level	Source[a]
		Minimum	Maximum	*a*	*b*		
	10	0.003	0.06	0.321	–0.36	AN	9
	8	0.00063	0.0039	0.141	–0.218	R	10
	13	0.00063	0.0039	0.257	–0.042	R	10
	18	0.00063	0.0039	0.384	–0.260	R	10
Rutilus rutilus	15	0.015	0.700	0.861	–0.22	S	26
	15	0.015	0.11	0.861	–0.035	AF	26
	20	0.016	0.704	1.16	–0.21	S	26
	20	0.016	0.130	1.60	–0.105	AF	26
	20	0.018	1.2	0.907	–0.22	R	4
	20	0.0016	0.0617	0.312	–0.07	R	2
	20	0.0011	0.068	0.881	–0.07	R	6
	20	0.0014	0.005	1.043	–0.041	S	7
	20	0.005	0.28	1.418	–0.232	S	7
	20	0.0014	0.28	1.579	–0.237	R	7
	20	0.0014	0.28	2.95	–0.156	AS	7
	20	0.0011	0.068	0.989	–0.06	AF	7
	20	0.001	0.068	1.33	–0.14	AF	7
	20	0.0024	0.720	1.19	–0.07	R	1
Salvelinus alpinus	2	0.05	0.124	0.127	–0.0855	R	27
Sparus aurata	19	0.000168	0.006	1.18	0	R	28
	24	0.000168	0.006	1.08	0.03	R	28
Stizostedion lucioperca	20	0.0008	0.079	0.220	–0.18	R	2
Theragra chalcogramma	6.2	0.000325	0.000786	0.322	–0.129	S	29
	6.2	0.000325	0.000786	0.295	–0.0301	R	29
	6.2	0.000286	0.000786	0.359	–0.0941	AF	29
	6.2	0.000286	0.000786	0.205	0.115	AF	29

Note: Metabolic allometry for 31 species of larval and juvenile teleost fishes. Species, experimental temperature, minimum and maximum mass of fish used in the experiments, allometric coefficients from $Vo_2 = aMASS^b$ with Vo_2 in units of mg O_2 g^{-1} h^{-1} and MASS in g wet weight, activity level (as presented in original references or interpreted from the methodology presented: AN, anaesthetized; S, standard; R, routine; A, active; AF, actively feeding; AS, actively swimming) and the original reference. Precision of estimates of mass and allometric coefficients are reported as presented in the references to a maximum precision of three significant digits.

[a]References: (1) Keckeis and Schiemer 1992; (2) Kudrinskaya 1969; (3) Houde and Schekter 1983; (4) Keckeis and Schiemer 1990; (5) Mishra and Singh 1979; (6) Wieser et al. 1988; (7) Kaufmann 1990; (8) Singh et al. 1982; (9) DeSilva and Tytler 1973; (10) Almatar 1984; (11) Dabrowski and Kaushik 1984; (12) Wieser and Medgyesy 1990*b*; (13) Kamler 1972; (14) Kamler 1976; (15) Kamler et al. 1987; (16) Laurence 1978; (17) Mukhamedova 1977; (18) Sheel and Singh 1981; (19) Hoss et al. 1988; (20) Edwards et al. 1969; (21) Spoor 1984; (22) Eldridge et al. 1982; (23) Wieser 1985; (24) Rombough 1988*b*; (25) Oikawa et al. 1991; (26) Wieser and Medgyesy 1990*a*; (27) Holeton 1973; (28) Quantz and Tandler 1982; (29) Yamashita and Bailey 1989.

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PERSPECTIVES

The prediction of hypolimnetic oxygen profiles: a plea for a deductive approach

David M. Livingstone and Dieter M. Imboden

Abstract: A simple deductive model, based on a general one-dimensional topographic vertical model, provides much insight into the behaviour of oxygen profiles in lake hypolimnia. The thickness of the anoxic layer is predicted to be directly proportional to the areal oxygen sink (sediment oxygen consumption) and inversely proportional to the difference between the oxygen concentration at the end of spring turnover and the reduction in oxygen concentration owing to the volume sink (water column oxygen consumption). Results from the model suggest that in highly eutrophic lakes, a small reduction in the oxygen concentration at the end of spring turnover could suffice to bring about a sudden, catastrophic expansion of the anoxic zone. In such lakes one must therefore be careful not to confuse effects of natural interannual meteorological variation or secular climate change with those of anthropogenic eutrophication or oligotrophication. A plea is made for a shift of approach away from the development of a multiplicity of inductive models, each with a limited range of applicability, for the prediction of hypolimnetic oxygen concentrations, toward the development of process-orientated, deductive models of more general applicability, especially in view of future problems in lake management likely to arise in the wake of climate change.

Résumé : Un simple modèle déductif, basé sur un modèle vertical topographique unidimensionnel général, fournit un aperçu important du comportement des profils d'oxygène dans l'hypolimnion des lacs. Selon les prévisions, l'épaisseur de la couche anoxique est directement proportionnelle à la désoxygénation par unité de surface (consommation d'oxygène dans les sédiments) et inversement proportionnelle à la différence entre la concentration d'oxygène à la fin du renversement printanier et la réduction de la concentration d'oxygène découlant de la désoxygénation par unité de volume (consommation d'oxygène dans la colonne d'eau). Selon les résultats obtenus avec le modèle, une faible réduction de la concentration d'oxygène à la fin du renversement printanier suffirait, dans les lacs très eutrophes, à provoquer un accroissement soudain et catastrophique de la couche anoxique. Il ne faut donc pas, dans de tels lacs, confondre les effets découlant d'une variation météorologique interannuelle naturelle ou d'un changement climatique séculaire et les effets d'une eutrophisation ou d'une oligotrophisation d'origine anthropique. On plaide en faveur d'une nouvelle approche, qui ne comporterait pas l'élaboration d'une multiplicité de modèles, possédant chacun un champ d'application limité, pour prévoir les concentrations d'oxygène dans l'hypolimnion, mais qui comprendrait plutôt l'élaboration de modèles déductifs, d'application plus générale et axés sur les processus, ceci étant particulièrement important face aux problèmes futurs qui risquent de survenir dans la gestion des lacs à la suite de changements climatiques.
[Traduit par la Rédaction]

Introduction

Both inductive and deductive models have important epistemological functions to fulfil in the sciences in general (Popper 1959) and in the environmental sciences in particular (Fig. 1). These functions are different but complementary. Inductive models are the concrete expression of an empirical data ordering and reduction process, the goal of which is the terse and organised description of data in terms of substantially fewer parameters than there are observations. The employment of inductive reasoning, arguing from the specific to the general, promotes the creation of new knowledge and understanding by assisting in the formulation of explanatory general principles based on a finite number of specific observations (Fig. 1, link A). Deductive models fulfil a completely different function. Since they employ deductive reasoning, arguing from general principles to specific cases, they cannot contribute directly to the creation of new knowledge, since the general principles involved are an integral part of such models (Fig. 1, link B). What they can do, however, is make deterministic predictions based on these general principles. Such predictions can be employed to test hypotheses and to investigate the completeness of a set of axioms relevant to a particular problem (Fig. 1, link C), or in the case of a complete and well-established set of general principles, predictions of the type of

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D.M. Livingstone[1] and D.M. Imboden. Department of Environmental Physics, Swiss Federal Institute of Environmental Science and Technology (EAWAG), and Department of Environmental Sciences, Swiss Federal Institute of Technology (ETH), Überlandstrasse 133, CH-8600 Dübendorf, Switzerland.

[1] Author to whom all correspondence should be addressed.
e-mail: living@eawag.ch

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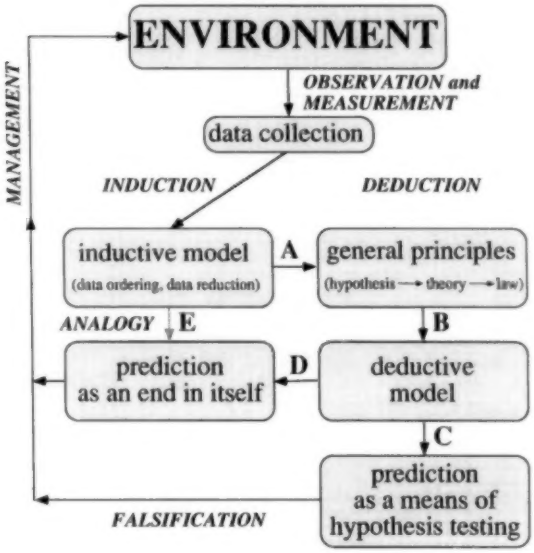
Fig. 1. Obtaining scientific knowledge: epistemology in the environmental sciences (cf. Popper 1959). A, the inductive formulation of explanatory general principles based on a finite number of specific observations; B, the concrete formulation of general principles to yield a deductive model; C, the employment of a deductive model for prediction purposes as a means of hypothesis testing; D, the employment of a deductive model for prediction purposes as an end in itself; E, the direct analogical use of inductive models for prediction purposes. Both the epistemological validity and practical use of the short-cut step E are disputed in the text.



interest to environmental managers can be made (Fig. 1, link D). Taking the short cut of employing inductive models directly for prediction purposes, i.e., without going through the stage of formulating a set of general principles in agreement with the large set of general principles already established in other contexts (Fig. 1, link E), is thus of dubious epistemological validity. Such empirical prediction, in effect arguing directly from the specific to the specific, rests essentially on analogy and is of necessity limited in its range of application.

One topic that has seen the development of a veritable plethora of predictive inductive models over the last two decades is oxygen depletion in lakes (e.g., Welch et al. 1976; Cornett and Rigler 1979, 1980; Charlton 1980; Jackson and Lasenby 1982; Trimbee and Prepas 1988; Cornett 1989; Molot et al. 1992). Inductive oxygen depletion models are based largely on curve fitting using multiple linear regression; some typical examples of the predictive equations that result are listed in Table 1. There is general agreement that one of the major factors affecting hypolimnetic oxygen depletion is lake morphometry; however, various functional forms for this dependence appear to yield statistically satisfactory results. The question therefore arises of which functional form best expresses the underlying behaviour of the system. By resolving the oxygen depletion rate into volumetric and areal components (e.g., Charlton 1980; Mathias and Barica 1980; Bossard and Gächter 1981), it is evident that the main morphometric effect involved in hypolimnetic oxygen depletion must be the linear dependence of the oxygen depletion rate $J(z)$ on the ratio $\alpha(z)$ of sediment area to water volume. In view of this, the reasons behind the selection of some of the functional relationships investigated by inductive modellers are not always clear. Cornett (1989), for example, did not investigate the linearity of the dependence of $J(z)$ on $\alpha(z)$ but on $1/\alpha(z)$ and $\ln(\alpha(z))$. In his final predictive equation (Table 1, eq. B), the morphometric effect was expressed rather obscurely in terms of the product of water temperature and $\ln(\alpha(z))$. Molot et al. (1992) did include a direct linear dependence of $J(z)$ on $\alpha(z)$ in their investigation but found that a logarithmic transformation of $J(z)$ gave better statistical results, and this was the functional form of the dependence finally chosen for their model (Table 1, eq. C). This illustrates one great danger involved in making an empirical choice between a variety of possible functional forms for the relationship between two (or more) variables: it is quite possible that several functional relationships exhibiting a degree of similarity over the range of data available may yield statistically significant results for a given data set. It is then a question of chance whether the functional relationship that explains the most variance is also the one matching the underlying behaviour of the system. If lack of knowledge of underlying processes precludes the development of a suitable deductive model, the use of a purely inductive model is justifiable. As will be shown, however, this may not be the case for hypolimnetic oxygen depletion.

The model

It has long been known that the chemistry of any given hypolimnetic water layer is determined to a great extent by the sediment area bounding the water layer horizontally at its edge (Alsterberg 1927; Hutchinson 1941; Hesslein 1980). This is because horizontal turbulent diffusion coefficients in the hypolimnion generally exceed vertical turbulent diffusion coefficients by several orders of magnitude (Imboden and Emerson 1978; Imboden and Joller 1984). The one-dimensional topographic vertical model introduced by Imboden and Emerson (1978) and fully described by Imboden and Joller (1984) is therefore a logical starting point for the deductive modelling of hypolimnetic oxygen profiles. The simplest version of this model for the O_2 depletion rate $J(z)$ at depth z (defined as positive downwards), where z is deep enough in the hypolimnion to allow the production and vertical transport of O_2 to be neglected, is

$$(1) \quad J(z) = -\frac{\partial C(z,t)}{\partial t} = J_V(z) + J_A(z)\,\alpha(z)$$

where $C(z,t)$ is the oxygen concentration at depth z and time t, $J_V(z)$ is the volume sink, or oxygen consumption rate associated with the water column, $J_A(z)$ is the area sink, or oxygen consumption rate associated with the sediments (essentially the horizontal flux of oxygen to the sediments), and $\alpha(z)$ is the morphometric function representing the ratio of sediment area to water volume at depth z, given by

$$(2) \quad \alpha(z) = -\frac{dA(z)}{dV(z)} = -\frac{1}{A(z)} \cdot \frac{dA(z)}{dz}$$

where $dV(z) = A(z)dz$ is the volume of the infinitely thin water

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Table 1. Examples of inductive model equations for the prediction of hypolimnetic oxygen depletion rates (A, B) and concentrations (C).

	Equation	Variables	Source	No. of lakes[a]
A	$AHOD = -277 + 0.5R_p + 5.0\bar{T}_H^{1.74} + 150 \ln(\bar{Z}_H)$	AHOD = mean areal hypolimnetic oxygen depletion rate (mg $O_2 \cdot m^{-2} \cdot d^{-1}$) R_p = areal phosphorus retention rate (mg $P \cdot m^{-2} \cdot yr^{-1}$) $\bar{T}_H$ = mean summer hypolimnetic temperature (°C) $\bar{Z}_H$ = mean thickness of hypolimnion (m)	Cornett and Rigler (1979)	12
B	$VOD = 9.87 + 0.0145T R_p + 7.82T - 2.50T \ln(V/SA)$	VOD = volume-related oxygen depletion rate in entire hypolimnia and in individual hypolimnion strata (mg $O_2 \cdot m^{-3} \cdot d^{-1}$) R_p = areal phosphorus retention rate (mg $P \cdot m^{-2} \cdot yr^{-1}$) T = water temperature (°C) V = water volume (m^3) SA = sediment area (m^2)	Cornett (1989)	29
C	$\log_{10}O_2(f)_z = 1.83 - 1.91/VSA_z - 7.06/O_2(i)_z - 0.0013TP_{so}^2$	$O_2(f)_z$ = end-of-summer oxygen concentration at depth z (mg $O_2 \cdot m^{-3}$) VSA_z = ratio of water volume to isobath area at depth z (m) $O_2(i)_z$ = beginning-of-summer oxygen concentration at depth z (mg $O_2 \cdot m^{-3}$) TP_{so} = total phosphorus concentration at spring turnover (mg $P \cdot m^{-3}$)	Molot et al. (1992)	32

[a]The number of lakes from which data were collected to formulate the equation.

layer at depth z. If required, an analytical expression for $\alpha(z)$ can be obtained by fitting the hypsographic curve $A(z)$ with a suitable empirical function. Imboden (1973) and Imboden and Joller (1984) suggest a function of the form

$$(3) \quad A(z) = A_0(1 - z/z_m)^q$$

where A_0 is the lake surface area, z_m the maximum lake depth, and q is a non-dimensional exponent that usually lies between 0.5 and 2.0. It follows that the morphometric function can be approximated by

$$(4) \quad \alpha(z) = q/(z_m - z)$$

In the simplest version of the model, J_V and J_A are considered to be constant with depth. A partial justification for this simplification is that the hypolimnion is approximately homothermic. If data from several water layers are available, the applicability of eq. 1 (with constant J_V and J_A) to the case of any one particular lake hypolimnion can be evaluated by testing the linearity of the relationship between $J(z)$ and $\alpha(z)$. If the relationship is found to be linear, the volume and area sinks are given by the intercept and gradient, respectively, of the regression line (Bossard and Gächter 1981).

Integrating eq. 1 over the whole hypolimnion reveals Charlton's (1980) equations for the morphometrical dependence of the mean volume-related and area-related hypolimnetic oxygen depletion rates (known as VHOD and AHOD, respectively, in the literature) to be a special case of the one-dimensional topographical vertical model:

$$(5) \quad VHOD = \frac{1}{V_h}\int_{z_h}^{z_m} J(z)\,dz = J_V + \frac{J_A}{\bar{z}_h}$$

$$(6) \quad AHOD = \frac{1}{A_h}\int_{z_h}^{z_m} J(z)\,dz = J_A + J_V\,\bar{z}_h$$

where z_h is the depth of the upper boundary of the hypolimnion, z_m the maximum depth of the lake, A_h the isobath area at $z = z_h$, V_h the volume of the hypolimnion, and $\bar{z}_h = V_h/A_h$ the mean thickness of the hypolimnion. Equation 6 shows why AHOD is not independent of lake morphometry (cf. Cornett and Rigler 1980; Molot et al. 1992), justifying the rejection of AHOD as an indicator of trophic status. Although AHOD may vary very little with hypolimnion thickness in shallow, eutrophic lakes, where most oxygen depletion is accounted for by the sediments ($J_A \gg J_V\,\bar{z}_h$), this will not be the case in deeper, more oligotrophic lakes in which a significant proportion of the total oxygen depletion occurs in the water column.

The hypolimnetic oxygen profile in summer

Aegerisee is a typical mesotrophic subalpine lake situated 724 m above sea level in the northern foothills of the Swiss Alps. Its hypsographic curve is represented well by eq. 3 with $q = 0.73$ (Fig. 2). Using weighted linear regression, the volume and area sinks were determined for the hypolimnion of this lake for the years 1951, 1975–1980, and 1982. The individual annual values of J_V did not differ significantly (t test, 95% confidence level) from the overall mean of 3.9 g $O_2 \cdot m^{-3} \cdot yr^{-1}$, a value that agrees almost exactly with that

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Fig. 4. Eighteen years of predicted end-of-summer hypolimnetic oxygen profiles $C(z)$ in Aegerisee (curves) compared with corresponding measured oxygen concentrations (●). Predicted profiles were calculated using eqs. 1 and 7 with a volume sink $J_V = 3.9$ g $O_2 \cdot m^{-3} \cdot yr^{-1}$ in all years, an area sink $J_A = 47$ g $O_2 \cdot m^{-2} \cdot yr^{-1}$ in 1950–1951 and $J_A = 113$ g $O_2 \cdot m^{-2} \cdot y^{-1}$ in 1968–1983, the Aegerisee morphometric function $\alpha(z)$ from Fig. 2, and the current value of the mean hypolimnetic oxygen concentration at the end of spring turnover for each year (C_{max}, open circle on abscissa).

Fig. 2. Hypsographic curve $A(z)$ of Aegerisee, obtained by fitting measured isobath areas (points) using eq. 3 ($z_m = 83$ m; $q = 0.73$), and the corresponding morphometric function $\alpha(z)$ obtained from eq. 4.

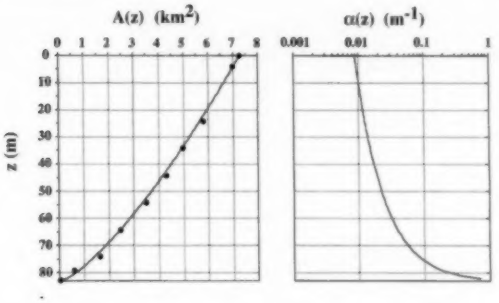
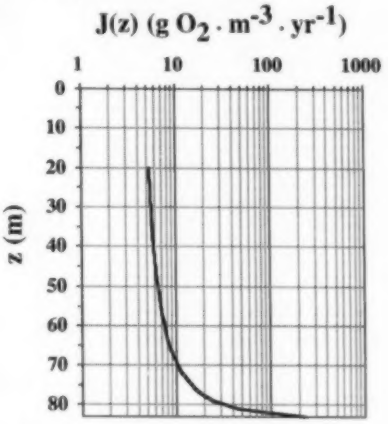


Fig. 3. Average theoretical oxygen depletion rate profile $J(z)$ for the Aegerisee hypolimnion ($z < 20$ m), obtained from eq. 1 with constant volume and area sinks $J_V = 3.9$ g $O_2 \cdot m^{-3} \cdot yr^{-1}$ and $J_A = 113$ g $O_2 \cdot m^{-2} \cdot yr^{-1}$, respectively.



found by Mathias and Barica (1980) for the oxygen consumption rate in the water column of Canadian lakes under ice irrespective of trophic status. In the case of the area sink J_A, only the value for 1951 (47 g $O_2 \cdot m^{-2} \cdot yr^{-1}$) differed significantly from the overall mean, the mean area sink during the remaining years being 113 g $O_2 \cdot m^{-2} \cdot yr^{-1}$. The increase in the area sink was presumably due to an increase in eutrophication between the 1950s and the 1970s that, in agreement with the findings of Mathias and Barica (1980), had no effect on the volume sink (Livingstone 1988). Setting $J_V = 3.9$ g $O_2 \cdot m^{-3} \cdot yr^{-1}$ and $J_A = 113$ g $O_2 \cdot m^{-2} \cdot yr^{-1}$ in eq. 1 yields an average theoretical O_2 depletion rate profile for Aegerisee (Fig. 3). Because of the rapid increase in $\alpha(z)$ with depth in the lower hypolimnion, the predicted net O_2 depletion rate increases much faster with depth there than it does in the upper hypolimnion, doubling from 30 to 70 m but doubling again from 70 to 78 m.

Equation 1 implies that the oxygen concentration $C(z)$ decreases linearly with time during summer stagnation. This has been confirmed in the field by Lasenby (1975) and by Cornett and Rigler (1984). Thus,

$$(7) \qquad C(z) = C_{max} - J(z)\delta t$$

where C_{max} represents the initial (uniform) hypolimnetic O_2 concentration at the end of spring turnover (10 April on average in Aegerisee) and δt the time elapsed since the end of spring turnover. In Fig. 4, 18 years of measured end-of-summer O_2 concentrations are compared with O_2 profiles predicted by eq. 7. In the computation of $J(z)$ from eq. 1, the value $J_V = 3.9$ g $O_2 \cdot m^{-3} \cdot yr^{-1}$ was used for all years; J_A was set to 47 g $O_2 \cdot m^{-2} \cdot yr^{-1}$ for 1950–1951 and to 113 g $O_2 \cdot m^{-2} \cdot yr^{-1}$ for all other years. With few exceptions (e.g., the upper hypolimnion in 1969), the predicted profiles agree well with the measured O_2 concentrations.

The end-of-summer hypolimnetic O_2 profile can therefore be predicted quite well from the initial hypolimnetic O_2 concentration at the end of spring turnover (C_{max}), the morphometric function ($\alpha(z)$), the volume sink (J_V), and the area sink (J_A). C_{max} is dependent mainly on ice cover (Livingstone 1993) and wind (Livingstone and Imboden 1993). Mathias and Barica (1980) showed that J_V tends to be constant over a large range of lakes of differing trophic status. The end-of-summer O_2 profile is therefore dependent mainly on the meteorological conditions prevailing during the previous winter, on the form of the lake basin, and on the rate of sediment O_2 consumption. This latter reflects the sedimentation of organic matter smoothed over several years (Granéli 1978; Mathias and Barica 1980) and hence in P-limited lakes is related to long-term P loading. It is therefore possible to model the hypolimnetic O_2 depletion rate as a function of P loading (Imboden 1973, 1974), which should allow indirect computation of the area sink if direct computation by regression is not possible.

In some years, measured and predicted oxygen concentrations do not agree perfectly in the deepest layers of the lake (Fig. 4). Since $\alpha(z)$, and thus also $J(z)$, is strongly depth-dependent close to the bottom of the lake, large vertical gradients in the oxygen concentration develop there. Vertical mixing by turbulent diffusion will tend to reduce the size of these large gradients (Imboden et al. 1983). In the model described here, the effect of this vertical mixing has been intentionally omitted for the sake of simplicity, and so $J(z)$ will tend to be overestimated in the deepest layers of lakes where the effect of mixing is greatest. Because of this, the model as it stands will presumably perform better in sheltered, deep lakes than in exposed, shallow lakes. While the employment of a combined reaction–mixing model may be appropriate for certain specific problems, such a model would require much more information about the lake and would hide some of the general features so nicely shown by the simple model described here.

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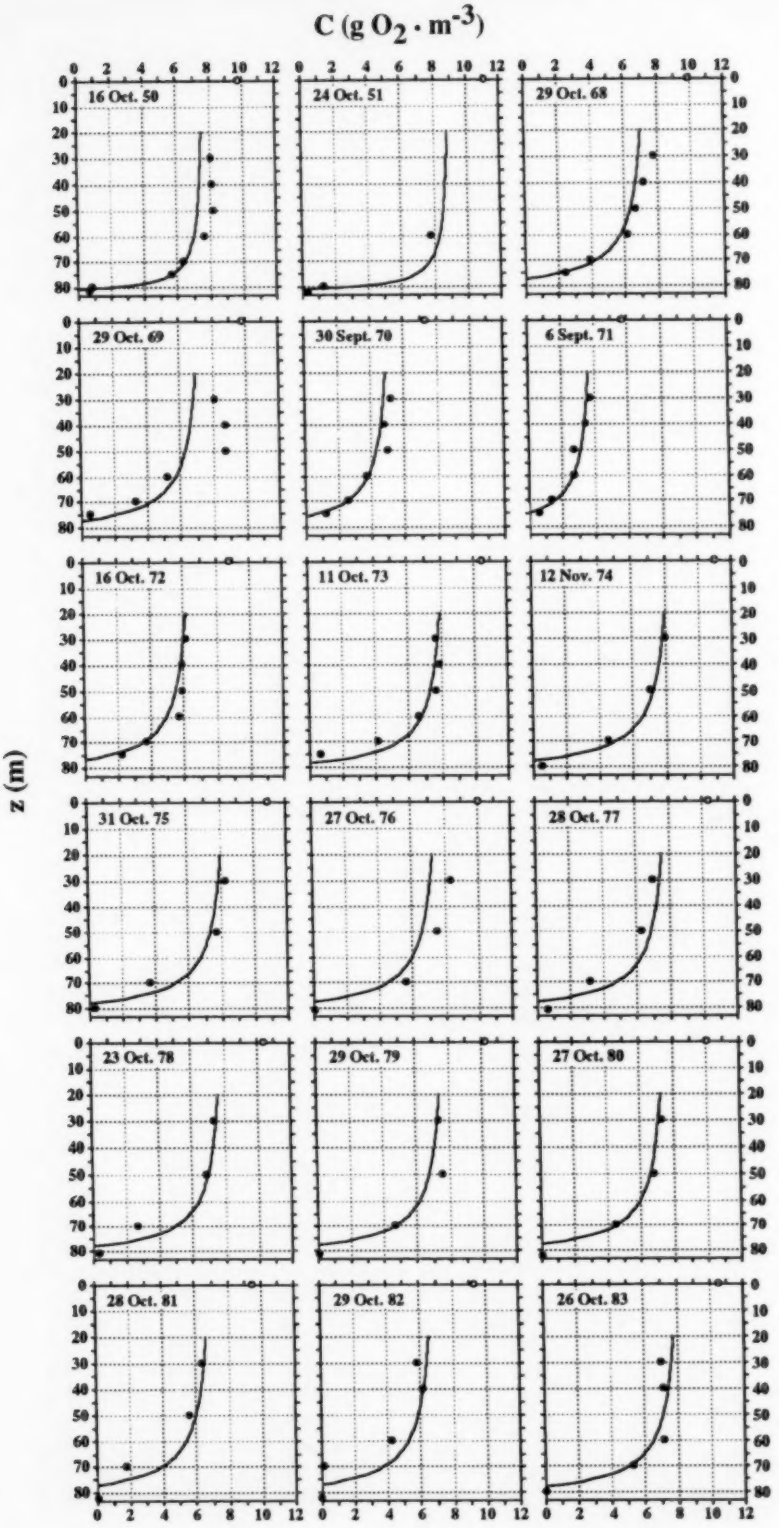

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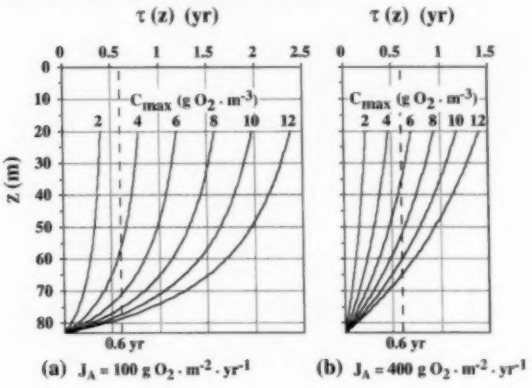

Fig. 5. Profiles of $\tau(z)$, the critical time to anoxia, for various values of C_{max} (the mean hypolimnetic oxygen concentration at the end of spring turnover). Two different trophic situations are illustrated: (*a*) the approximate situation pertaining in Aegerisee in 1975–1983, with an areal oxygen sink $J_A = 100$ g $O_2 \cdot m^{-2} \cdot yr^{-1}$ and (*b*) a hypothetical situation in the same lake with $J_A = 400$ g $O_2 \cdot m^{-2} \cdot yr^{-1}$, a value similar to that found in neighbouring hypereutrophic lakes.

The critical time to anoxia and vertical extent of the anoxic layer

Barica and Mathias (1979) introduced the concept of the critical time required for an ice-covered lake to reach total anoxia. Building on their idea, we can define the critical time to anoxia $\tau(z)$ for any water layer in a stratified hypolimnion in summer as

$$\tau(z) = C_{max}/J(z) \quad (8)$$

If the critical time at depth z is exceeded by the duration of summer stagnation, then the lake is likely to be anoxic at this depth at the end of summer. Taking Aegerisee as an example, summer stagnation lasts about 0.6 yr on average, and C_{max} can vary within the range 6–11 g $O_2 \cdot m^{-3}$. Thus, on the basis of the profiles of critical time for various values of C_{max} (Fig. 5*a*), anoxia is predicted to be confined to the region below 75 m depth in normal years, in which C_{max} exceeds 9 g $O_2 \cdot m^{-3}$, and to extend upwards a further 5 m in years such as 1970 and 1971, in which C_{max} was considerably lower than usual (owing to unusually long periods of ice cover). The oxygen measurements (Fig. 4) confirm that this is indeed the case.

Because of the shape of the morphometric function $\alpha(z)$ (Fig. 2) and the corresponding shape of the net O_2 depletion rate profile $J(z)$ (Fig. 3), the critical time $\tau(z)$ increases much less rapidly with increasing height above z_m in the central and upper hypolimnion than in the lower hypolimnion (Fig. 5*a*). Thus, in mesotrophic or slightly eutrophic lakes like Aegerisee, in which anoxia is normally confined to the lower hypolimnion, small variations in C_{max} will have little effect on the vertical extent of the end-of-summer anoxic layer. In contrast, in highly eutrophic lakes in which the anoxic layer regularly extends upwards into the region in which $\tau(z)$ varies little with depth, small year-to-year variations in C_{max} will have a disproportionately large effect on the extent of the anoxic layer. This is illustrated well by comparing Aegerisee in the 1980s (with $J_A = 100$ g $O_2 \cdot m^{-2} \cdot yr^{-1}$; Fig. 5*a*), with a hypothetical highly eutrophic Aegerisee (with J_A quadrupled to 400 g $O_2 \cdot m^{-2} \cdot yr^{-1}$, a value similar to that actually found in hypereutrophic Swiss lakes such as Baldeggersee; Fig. 5*b*). A quite realistic between-year decrease in C_{max} from 10 to 6 g $O_2 \cdot m^{-3}$ is predicted to result in a relatively small expansion of the anoxic zone in the slightly eutrophic case (from 6 to 12 m in thickness; Fig. 5*a*) but in a much greater expansion in the highly eutrophic case (from 23 to 48 m in thickness; Fig. 5*b*). Thus, an unfavourable constellation of meteorological factors can cause a reduction in C_{max} that could suffice to bring about a sudden, catastrophic expansion of the anoxic zone in a highly eutrophic lake. In such lakes one must therefore be careful not to confuse the short-term effects of fluctuations in C_{max} (due largely to natural interannual meteorological variation) with the long-term effects of anthropogenic eutrophication or oligotrophication.

This last point is of particular interest when considering the possible long-term effects of global warming on natural lake oxygenation. The duration of ice cover, which is a major factor influencing C_{max} in strictly dimictic lakes (Livingstone 1993), is undergoing a long-term decrease as a result of global warming (D.M. Livingstone, unpublished results). This will, on the one hand, result in a corresponding long-term increase in C_{max}, leading to a tendency for the anoxic layer to contract. On the other hand, global warming will also result in a lengthening of the period of summer stagnation (by more than a month in deep and medium-deep lakes according to Hondzo and Stefan (1993), i.e., from 0.6 to 0.7 yr in Fig. 5), which will result in a tendency for the anoxic layer to expand. There is a danger of the consequences of such effects being misinterpreted as being due to the success or failure of measures introduced to combat eutrophication. Keeping this in mind, it would be useful to be able to predict the extent of the anoxic layer as a function of C_{max}, δt, and the two oxygen consumption rates J_V and J_A. Neglecting for the moment vertical turbulent diffusion, the thickness of the anoxic layer at the end of summer, or indeed at any time during the previous summer stagnation, can be easily estimated using the critical time concept. At the upper boundary of the anoxic layer ($z = z_a$),

$$\tau(z_a) = \delta t \quad (9)$$

From eqs. 1, 8, and 9, it follows that

$$\alpha(z_a) = \frac{C_{max} - J_V \delta t}{J_A \delta t} \quad (10)$$

After computing the right-hand side of eq. 10, the value of z_a can be read off a plot of the morphometric function against depth. Alternatively, eq. 4 can be employed to give an analytical expression for z_a:

$$z_a = z_m - \frac{q J_A \delta t}{C_{max} - J_V \delta t} \quad (11)$$

From eq. 11, the thickness of the anoxic layer ($z_m - z_a$) can be seen to be directly proportional to the O_2 depletion $J_A \delta t$ due to the area sink and inversely proportional to the difference ($C_{max} - J_V \delta t$) between the spring hypolimnetic O_2 concentration and the depletion in the hypolimnetic O_2 concentration due to the volume sink. The depth z_a of the upper boundary of the anoxic layer is therefore linearly dependent on the area sink,

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$$(12)\quad \frac{\partial z_a}{\partial J_A} = -\frac{q\,\delta t}{C_{max} - J_V \delta t}$$

but the dependence of z_a on the spring hypolimnetic O_2 concentration C_{max} is non-linear:

$$(13)\quad \frac{\partial z_a}{\partial C_{max}} = -\frac{q\,J_A\,\delta t}{(C_{max} - J_V \delta t)^2}$$

In a well-aerated, oligotrophic lake (C_{max} high, J_A low), $\partial z_a/\partial C_{max}$ is small and the thickness of the anoxic layer is insensitive to fluctuations in the maximum spring hypolimnetic O_2 concentration that may occur from year to year. However, in a badly aerated, eutrophic lake (C_{max} low, J_A high), $\partial z_a/\partial C_{max}$ is large and the thickness of the anoxic layer is extremely sensitive to such fluctuations.

Equation 11 was used to compute the theoretical dependence of the depth of the upper boundary of the anoxic layer on the mean hypolimnetic O_2 concentration at the end of spring turnover and the O_2 area sink for Aegerisee (Fig. 6). The existence of vertical turbulent diffusion in the lower hypolimnion will tend to increase O_2 concentrations there above predicted levels, so that eq. 11 will overestimate the thickness of a thin anoxic layer, giving a worst case scenario (the thicker the anoxic layer is, the less the overestimate will be). It is clear that in Aegerisee, the combined effect of the spring hypolimnetic O_2 concentration and the O_2 area sink could not have allowed anoxia to occur above 70 m in any of the 19 years for which data are available (Fig. 6), but that an increase in the magnitude of the O_2 area sink would make the thickness of the anoxic zone extremely sensitive to the degree of aeration occurring during spring turnover.

There is a theoretical upper limit to C_{max} that may be important in some lakes. This upper limit (see Fig. 6) is the steady-state O_2 concentration C_∞, given by

$$(14)\quad C_\infty = C_s - J\bar{z}/\beta$$

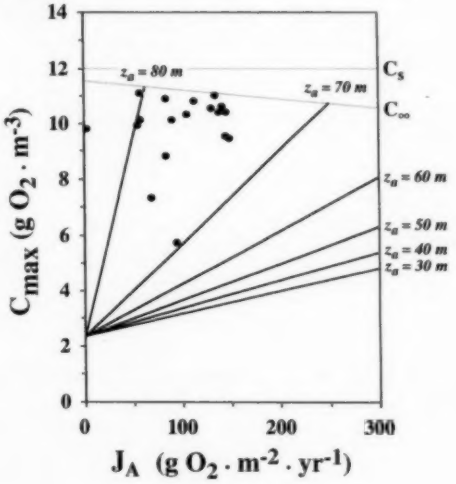
where C_s is the concentration of O_2 in aqueous solution in Henry's law equilibrium with atmospheric O_2 (i.e., the "O_2 saturation concentration"), J the mean net O_2 depletion rate in the lake, $\bar{z}$ the mean lake depth, and β the gas transfer coefficient, all at spring turnover (Livingstone 1993; Livingstone and Imboden 1993). In Aegerisee, $\bar{z} = 48.9$ m and mean values of J and β (for 1974/75 to 1983/84) are 5.5 g $O_2 \cdot m^{-3} \cdot yr^{-1}$ and 0.82 $m \cdot d^{-1}$, respectively (Livingstone 1993). Thus, in Aegerisee the equilibrium deficit $C_s - C_\infty$ is usually less than 1 g $O_2 \cdot m^{-3}$. In more wind-protected (low β) or more eutrophic (high J) lakes, however, the depression of C_∞ with respect to C_s will be of greater importance.

A plea for a deductive approach

Despite its simplicity, the model described above yields surprisingly good predictions of hypolimnetic oxygen profiles. However, the specific form of the model may be less important than its function as a straightforward example of a deductive approach to the problem.

The rôle assigned to prediction in the environmental sciences depends largely on the philosophical convictions held by the investigator (cf. Lehman 1986 with Peters 1986). Two main schools of thought in the environmental sciences can be distinguished. The first, which considers understanding to be the ultimate aim of environmental research, views *prediction as a tool in hypothesis testing.* The second, which considers environmental management to be of paramount importance, reverses the situation and views *hypotheses as tools for prediction.*

Fig. 6. Theoretical dependence of the depth z_a of the upper boundary of the anoxic layer (lines) in Aegerisee on the mean hypolimnetic oxygen concentration at the end of spring turnover (C_{max}) and the oxygen area sink (J_A) (from eq. 11 with $J_V = 3.9$ g $O_2 \cdot m^{-3} \cdot yr^{-1}$ and $\delta t = 0.6$ yr). Values of C_{max} and J_A for Aegerisee are shown on the same plot (●: 1950–1951; 1953; 1968–1983). The oxygen saturation concentration C_s during spring turnover is 12 g $O_2 \cdot m^{-3}$ in Aegerisee. The steady-state oxygen concentration C_∞ (eq. 14), shown as a function of J_A, represents a theoretical upper limit to C_{max}.



In the first school of thought, according to the traditionally accepted view of scientific methodology as formulated by Popper (1959), a general hypothesis concerning a system is falsified or corroborated by using the hypothesis deductively to predict the behaviour of the system under a set of specific conditions and comparing the resulting predicted state of the system with observations or measurements of the state of the system under the same set of specific conditions (Fig. 1). The ultimate goal of this procedure, an increased subjective understanding of the system, can be viewed as a simplified mapping of the system on to the mind of the investigator. A deductive model is a formal expression of the communicable aspects of this subjective understanding; tests of predictions made by deductive models are therefore indirect tests of the understanding on which they are based. Because it is a description of measurable variables and not the formal expression of a hypothesis, an inductive model cannot, logically, be used for hypothesis testing in this way (see Fig. 1). The epistemological function of an inductive model is not the testing of randomly formulated ad hoc hypotheses, but rather the discovery of statistical relationships that may lead to the formulation of testable hypotheses, or as Lehman (1986) put it, to open paths of creative thought.

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The second school of thought regards hypotheses and theories as of value only insofar as they are capable of delivering statistically accurate predictions. This instrumentalist view (Popper 1983) has been adopted under the name of "predictive" or "empirical" limnology by limnologists who see "theories in limnology as useful instruments to describe, manipulate, and control our environment" and who reject a "subjective feeling of 'understanding' as an end in itself" (Peters 1986). From this standpoint, the question at issue in comparing an inductive with a deductive approach is a practical one: which has the greater predictive value?

Deductive prediction is by inference; inductive prediction is by analogy (Fig. 1). Because it is based on analogy, the validity of an inductive model is confined to the range of data used in its formulation. This reduces its general predictive value by putting spatial and temporal limits on its applicability. In a situation of secular climatic change, in which future system behaviour may be determined by constellations of forcing factors never before encountered, a deductive approach to prediction may become imperative.

Since the predictive failure of an inductive model does not falsify any underlying hypothesis (see Fig. 1), no explanation can be given for discrepancies between model predictions and observations. The consequences of predictive failure are therefore limited to rejection of the specific model concerned. In contrast, disagreement between observations and the predictions of a deductive model forces one to examine the validity and completeness of the set of premises on which the model is based and to reject, revise, or supplement them accordingly. In fact, it is even possible to predict cases in which deductive models will perform badly or fail. Thus, even if an inductive model yields smaller discrepancies between predicted and observed values than does a well-founded, process-orientated deductive model for a specific, limited range of data, in the general case its predictive value may well be inferior to that of the deductive model.

The deductive approach requires premises rooted in the wider field of scientific knowledge, whereas the inductive approach does not. Thus, although empirical limnologists frequently give the impression of adhering strictly to Popperian method, their approach more closely corresponds to Feyerabend's (1988) description of scientific *practice* (as opposed to *method*), in which the Popperian distinction between the contexts of discovery (via induction) and justification (via deduction) is abolished. Feyerabend (1988) explicitly defends the use of ad hoc hypotheses produced inductively on the grounds that strict adherence to Popperian method would make scientific progress impossible. In contrast to the impression given by proponents of both the deductive and inductive approaches within the field of limnology, the deductive approach to prediction is therefore essentially traditionalist and conservative, whereas the inductive approach, which has the potential to break new ground by calling established knowledge and accepted principles into question, is essentially revolutionary in nature. This latter quality, although necessary to scientific progress in the long term, is unlikely to enhance predictive value in the short term: predictions based on all available information, including knowledge of relevant processes obtained in other contexts, are likely to be of most value. While successful inductive models can challenge established ideas, preventing stagnation on the frontiers of science, they may not always be the most appropriate tool for predictive purposes.

Thus, irrespective of whether understanding or predictive power is regarded as being of higher priority, a deductive approach to the modelling of hypolimnetic oxygen concentrations is probably preferable to the more frequently employed inductive approach. Instead of investing resources in the development of a multiplicity of locally applicable inductive models, it may be more profitable in future to direct more effort into process-orientated research. The resulting increase in knowledge could then serve as a basis for developing a more generally applicable deductive model to perform the same function as the various inductive models now in use. This approach may be preferable even if the predictions made by such a deductive model turn out in certain cases to be less accurate statistically than short-cut predictions made by inductive models based on limited data. Such a shift in approach may be made particularly necessary by future problems in lake management likely to arise in connection with climate change, since past behaviour may not be a good guide to future behaviour under conditions in which meteorological forcing factors exhibit secular trends. The best approach is likely to be provided by combining a deductive, process-orientated basis with a minimum of inductive elements describing processes that are not yet well enough understood to be modelled otherwise.

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DISCUSSION

Comment — Estimation of selection differentials from fish scales: a step towards evaluating genetic alteration of fish size in exploited populations[1]

A.K. Sheridan

As discussed by Falconer (1989, pp. 319, 320), Miller and Kapuscinski (1994) estimated a correlated selection differential (S'_y) for the trait length of scale radius (trait y) to selection for some component of fish size (trait x) and not the actual selection differential (S_x) for the selected trait (trait x). Thus, it is not possible to use the following formula to estimate response in the selected trait:

$$R = h^2 S$$

(i.e., $R_x = h_x^2 S_x$). By substituting $S'_y \sigma_{p(x)} / r_p \sigma_{p(y)}$ for S_x (Falconer 1989), we get

$$R_x = h_x^2 S'_y \sigma_{p(x)} / r_p \sigma_{p(y)}$$

where S'_y and S_x are as defined above, R_x is the response in the selected trait (trait x), h_x^2 is the heritability for trait x, r_p is the phenotypic correlation between traits x and y, $\sigma_{p(x)}$ is the phenotypic standard deviation for trait x, and $\sigma_{p(y)}$ is the phenotypic standard deviation for trait y.

As we do not have a precise knowledge of what trait x actually is (fish width?), it is not possible to estimate either its heritability or phenotypic standard deviation or the phenotypic correlation between it and length of scale radius.

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A.K. Sheridan. NSW Fisheries Research Institute, Cronulla, NSW 2230, Australia. e-mail: sheridab@fisheries.nsw.gov.au

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Reply — Estimation of selection differentials from fish scales: a step towards evaluating genetic alteration of fish size in exploited populations[1]

L.M. Miller and A.R. Kapuscinski

Sheridan (1996) has correctly noted that we cannot use the model $R = h^2S$ to quantitatively predict changes in fish size on the basis of selection differentials imposed on the correlated trait, length of scale radius. Furthermore, uses of this model in previous publications to predict response or estimate heritabilities of fish weight from fishing data are also invalid. Unlike human application of intentional selection in a breeding program, fishing gear does not measure the same trait on each fish and keep or discard fish on the basis of its value. Therefore, any size trait one might choose to examine is correlated, although possibly quite closely, with the unknown trait x, the basis of gear selectivity. Thus, the heritability estimates for the weight of pink salmon by Ricker et al. (1978) are in error. They based their estimates on a rearrangement of the above model to $h^2 = R/S$, where R is the measured change in fish weight. Similarly, the proposed experimental design by McAllister et al. (1992) to estimate the heritability of fish weight is also in error. The fundamental error in both cases is the same: fish weight is only correlated with the unknown trait that determined whether or not fish were caught. As discussed by Falconer (1989), it is not possible to estimate the heritability of a correlated character without knowing the genetic correlation between it and the selected trait. However, it is likely that strong positive genetic and phenotypic correlations are present between fish weight and the unknown selected trait. Finally, Rowell et al. (1989) should have clearly indicated that they were estimating correlated selection differentials when they used otoliths in a manner similar to our use of scales.

The corrected formula presented by Sheridan can still be used to qualitatively indicate the risk of genetic selection by fishing gear and predict its direction (i.e., selection for smaller or larger fish size). Using it to qualitatively predict risk of selection, however, requires more assumptions than we previously presented (see section entitled S_0 as a genetic risk management tool, in Miller and Kapuscinski (1994)). For the direct selection model, we assumed that heritabilities of fish size traits are positive, i.e., $h^2 > 0$. If h^2 is positive in this model, then the direction of the predicted selection response is the same sign as the selection differential. To predict the direction of response with Sheridan's (1996) correction, phenotypic standard deviations for scale and fish size also must be greater than zero and the sign of the phenotypic correlation between them must be known. Stated otherwise, predicting the direction of response requires that the regression coefficient of fish size on scale size must be non-zero. The general phenomenon of a positive relationship between fish size traits (often length) and scale size is well established and has been the basis of numerous fish growth studies (Carlander 1987). Although specific fish size traits cannot be used for direct estimation of response to selection by a particular fishing gear or method, it is reasonable to assume that the regression of the unknown fish size trait on length of scale radius is also positive. The direction of the selection differential for the unknown fish size trait (S_x) would therefore be the same as for the correlated selection differential on scale size (S'_y). Then, assuming the heritability of fish size to be greater than zero, the predicted response to selection will be of the same sign as the correlated selection differential.

Sheridan (1996) has shown that we cannot use the genetic selection model to directly predict changes in fish size on the basis of gear selectivity. Although we agree with this limitation, our main point is that measurement of S'_y alone remains a worthwhile way of monitoring for the risk of genetic alteration owing to selective fisheries. The exact magnitudes of genetic change in fish size may not be predictable, but agreements between the directions and relative magnitudes of correlated selection differentials and corresponding changes in fish size will provide strong inference for selective fishing as a force of genetic change.

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L.M. Miller and A.R. Kapuscinski. Department of Fisheries and Wildlife, University of Minnesota, 200 Hodson Hall, 1980 Folwell Avenue, St. Paul, MN 55108-1036, U.S.A.

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Commentary on Sheridan (1996) and Miller and Kapuscinski (1996) concerning "Estimation of selection differentials from fish scales: a step towards evaluating genetic alteration of fish size in exploited populations"[1]

Murdoch K. McAllister and Darren M. Gillis

At issue is the elucidation of appropriate methods to assess genetic changes from selective fishing as discussed in Sheridan (1996) and Miller and Kapuscinski (1996). We discuss a possible error in Sheridan's (1996) equation for predicting a response in a trait directly acted upon by selection from an apparent selection differential in a trait that is correlated with the directly selected trait. We also suggest that Miller and Kapuscinski (1996) overextend Sheridan's (1996) critique regarding equations used to predict responses of traits affected by selective fishing. We conclude by reaffirming that quantitative approaches to assessing genetic and other changes of selective fishing are both desirable and appropriate.

Response to Sheridan (1996)

Sheridan (1996) quite correctly used Falconer (1989) to point out a difficulty with the approach in Miller and Kapuscinski (1994). According to Falconer (1989), the following formula for a correlated response in a trait, y, which is correlated with some trait, x, that is directly acted upon by selection is incorrect:

$$(1) \quad R'_y = h_y^2 S'_y$$

where R'_y is the response in the trait y that is correlated with the directly selected trait x, h_y^2 is the heritability of the correlated trait y, and S'_y is the "correlated" selection differential of the correlated trait, y. This is because the correlated selection differential, S'_y, is only an apparent selection differential (i.e., it may not actually be caused by, or related to, the agent of selection). The use of correlated selection differentials should only be considered when it is known that they were caused by the agent of selection (e.g., size-selective fishing). It is therefore not possible to use eq. 1 and observations of R'_y and S'_y to assess the magnitude of responses in the selected trait, R_x (Sheridan 1996).

However, there also appears to be an error in Sheridan's (1996) equation that expresses the response in a selected trait as a function of the selection differential of a correlated trait:

$$(2a) \quad R_x = \left(h_x^2 \frac{\sigma_{P,x}}{r_P \sigma_{P,y}} \right) S'_y$$

where h_x^2 is the heritability of the selected trait, r_P is the phenotypic correlation between the correlated and selected traits, and $\sigma_{P,y}$ and $\sigma_{P,x}$ are the phenotypic standard deviations in the correlated and selected traits, respectively. Equation 2a must be wrong because it states that the smallest phenotypic correlations (i.e., those approaching 0) between the selected and correlated traits will result in the strongest relationship between the response of the selected trait and the selection differential in the correlated trait.

We suggest that a correction to Sheridan's equation can be obtained by the following steps. As with Sheridan (1996), we start with the equation:

$$(2b) \quad R_x = h_x^2 S_x$$

where S_x is the selection differential of the selected trait x (Falconer 1989). If we have only a value for the selection differential of the correlated trait, S'_y, then we would like to replace S_x in eq. 2b with the expected value of S_x given the observed value for S'_y, $E(S_x|S'_y)$. Thus, equation 2b becomes

$$(2c) \quad R_x = h_x^2 E(S_x|S'_y)$$

Note that $E(S_x|S'_y)$ is not obtainable from rearranging the expression for the expected value of S'_y given S_x (i.e.,

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M.K. McAllister[2] and D.M. Gillis.[3] School of Resource and Environmental Management, Simon Fraser University, Burnaby, BC V5A 1S6, Canada.

[1] Comment by A.K. Sheridan. 1996. Can. J. Fish. Aquat. Sci. **53**: 933. Reply by L.M. Miller and A.R. Kapuscinski. 1996. Can. J. Fish. Aquat. Sci. **53**: 934–935.

[2] Author to whom all correspondence should be sent at the following address: Renewable Resource Assessment Group, Imperial College, 8 Princes Gardens, London, SW7 1NA, U.K. e-mail: mmmcal@ic.ac.uk

[3] Present address: Department of Zoology, Duff Robin Building, University of Manitoba, Winnipeg, MB R3J 2N2, Canada.

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$E(S'_y|S_x) = S_x r_P \sigma_{P,y}/\sigma_{P,x})$ as in Sheridan (1996). Instead, $E(S_x|S'_y)$ can be obtained from the regression of S_x on S'_y:

(2d) $$E(S_x|S'_y) = b_{P,x,y} S'_y$$

where $b_{P,x,y}$ is the slope of the regression of S_x on S'_y:

(2e) $$b_{P,x,y} = \frac{\mathrm{cov}_{P,x,y}}{\sigma^2_{P,y}}$$

and $\mathrm{cov}_{P,x,y}$ is the phenotypic covariance between traits x and y (Falconer 1989). Given that:

(2f) $$\mathrm{cov}_{P,x,y} = r_P \sigma_{P,x} \sigma_{P,y}$$

(Falconer 1989), then

(2g) $$E(S_x|S'_y) = r_P \frac{\sigma_{P,x}}{\sigma_{P,y}} S'_y$$

By combining eqs. 2c and 2g,

(2h) $$R_x = \left(h_x^2 r_P \frac{\sigma_{P,x}}{\sigma_{P,y}} \right) S'_y$$

Equation 2h makes sense intuitively because it suggests that the highest phenotypic correlations (i.e., those approaching 1) will result in the strongest relationship between the selected trait and the correlated selection differential.

As suggested above, use of eq. 2h would be sensible only if S'_y were known to be caused by selective fishing (i.e., r_P was known to be high). A further problem with predicting R_x from S'_y, as Miller and Kapuscinski (1996) point out, is that it may be difficult to identify the actual trait or traits selected by fishing gear. If they were identified, the selected and correlated traits would have to be accurately and precisely measured, which might be very difficult and (or) expensive.

The possibility of using apparent selection differentials in scale radius length to assess genetic changes from selective fishing (Miller and Kapuscinski 1994) appears to be rather dubious. Recall that r_P in eq. 2h should be large, e.g., >0.8, for this equation to be useful. However, for walleye (*Stizostedion vitrium*), estimates of r_P between fish size (dimension not specified) and scale radius length are rather low (i.e., averaging about 0.5) and highly variable among samples (ranging between 0.1 and 0.9) (Miller and Kapuscinski 1994).

Response to Miller and Kapuscinski (1996)

Miller and Kapuscinski (1996) quite appropriately indicate the limitations of Miller and Kapuscinski (1994). However, we dispute Miller and Kapuscinski's (1996) sweeping statement that uses of eq. 1 in previous publications to predict responses of fish weight to selective fishing from fishery data are "invalid" and have a "fundamental error." We also suggest that advocacy of only a qualitative approach to assessing genetic change appears to be unduly limiting.

There are, however, a few technical points in Miller and Kapuscinski (1996) with which we agree. We agree that, in the strictest sense, heritability of a correlated trait, h_y^2 (Falconer 1989), cannot be estimated from eq. 1 using estimates of responses, R'_y, and selection differentials, S'_y, in the correlated trait. We also agree that in Ricker et al. (1978) and McAllister et al. (1992) body weight of adult pink salmon (*Oncorhynchus gorbuscha*) is a "correlated" trait, not a "selected" trait. The main trait selected by gill net fishing gear in adult salmon appears to be girth (Todd and Larkin 1971; Wilson and Pearse 1984; Wilson and Andrews 1987). We also agree that body weight in adult pink salmon is likely to be very closely related (phenotypically and genetically via growth rate genes) to traits selected by fishing gear (e.g., girth).

We suggest, however, that an equation analogous to eq. 1 (eq. 7) can be used to quantitatively assess and predict responses in traits that are highly correlated, phenotypically and genetically, with selected traits. Falconer (1989, p. 318) provides the following relationship between a correlated response and the selection differential in the directly selected trait:

(3) $$R'_y = i h_x h_y r_A \sigma_{P,y}$$

where R'_y is the response for the trait y, which is correlated with the directly selected trait x, h_x and h_y are the square roots of heritability for traits x and y, respectively, r_A is the genetic correlation coefficient between traits x and y, i is the selection intensity on trait x, and $\sigma_{P,y}$ is the phenotypic standard deviation in trait y. This equation can be rewritten as

(4) $$R'_y = h_x h_y r_A S_x \frac{\sigma_{Py}}{\sigma_{Px}}$$

since

(5) $$i = \frac{S_x}{\sigma_{Px}}$$ (Falconer 1989)

Equation 4 is potentially useful in fisheries analysis because the interest may only be in a correlated trait that has biological and economic significance (e.g., body weight) (Ricker et al. 1978). However, the trait that is directly selected by fishing gear may not always be precisely known. Furthermore, data may be available only for the correlated trait. It would therefore be of interest to express the correlated response, R'_y, as a function of the correlated selection differential, S'_y. However, as noted above, such an approach would only be sensible if the apparent selection differential in the correlated trait, S'_y, was known to be caused by selective fishing. Using equations 4 and 2g, which expresses the expected value for S_x given S'_y, and then rearranging gives

(6) $$R'_y = (r_A r_P h_x h_y) S'_y$$

or

(7) $$R'_y = h^2_{y,x} S'_y$$

where

(8) $$h^2_{y,x} = r_A r_P h_x h_y$$

and $h^2_{y,x}$ may be roughly interpreted as the "apparent heritability" for trait y when selection acts directly on trait x. Equation 7 appears to be correct because it indicates that the highest heritabilities of traits x and y and the highest genotypic and phenotypic correlations between traits x and y will result in the strongest relationship between the correlated response and the correlated selection differential. Equation 7 also suggests that

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correlated selection differentials will only be useful for predicting R'_y in instances in which the correlated and selected traits are highly correlated, phenotypically and genetically.

Some useful identities can be defined when limiting conditions are considered. For example, if the two characters x and y were the same, then $r_A = r_P = 1$ and

$$(9) \quad h_x^2 = h_y^2 = h_{y,x}^2$$

Hence, as the genetic and phenotypic correlations between traits x and y approach 1, the values for h_x^2, h_y^2, and $h_{y,x}^2$ would be expected to increase in similarity:

$$(10) \quad \text{As } r_A \to 1 \text{ and } r_P \to 1, \text{ then } h_x^2 \to h_y^2,\ h_{y,x}^2 \to h_x^2, \text{ and } h_{y,x}^2 \to h_y^2$$

Under such conditions, eq. 7 would approach identity with eqs. 1 and 2b.

Falconer (1989, p. 320) observed that "without knowing the genetic correlation r_A, it is not possible to use the correlated selection differential [S'_y] either to estimate the heritability of character [y] or to predict the correlated response." That it is not possible to use S'_y to estimate h_y^2 without knowing r_A (and h_x and r_P) is perfectly clear. However, if the joint quantity represented by $h_{y,x}^2$ is known and r_A and r_P are known (or assumed) to be high (say >0.8), prediction of R'_y with S'_y is possible. An important issue, therefore, is whether the quantity $h_{y,x}^2$ is estimable from observations of R'_y and S'_y. By regressing observations R'_y on observations S'_y (e.g., McAllister et al. 1992) it is clearly possible to estimate the quantity $h_{y,x}^2$.

The use of eq. 7 avoids the potential difficulties of having to precisely measure the selected trait or traits in selection experiments in the field. Instead a trait may be chosen that is (*i*) of interest from conservation and economic considerations, (*ii*) highly correlated (genotypically and phenotypically) with the selected trait, and (*iii*) inexpensive and easy to measure. The interpretation of results of the second procedure appears to be straightforward. For example, a small, precise estimate of $h_{y,x}^2$, say <0.1, would imply that there is little statistical relationship between the correlated response and the correlated selection differential, and that selective fishing will cause little observable change in the correlated trait y between one generation and the next.

In applications of eq. 1 to fisheries situations, the main interests have included (*i*) assessing whether historic changes in the "correlated" trait, body weight, could have been caused by genetic selection from size-selective fishing (Ricker et al. 1978), (*ii*) evaluating the potential statistical performance of proposed size-selective fishing experiments that could more effectively test for a genetic effect of size-selective fishing (McAllister et al. 1992), and (*iii*) predicting the potential responses in body weight to size-selective fishing in the future if the genetic selection hypothesis were true (McAllister and Peterman 1992). Genetic responses in traits directly selected by fishing (i.e., girth) were not of direct interest. Observations of R'_y and S'_y in body weight were obtainable; observations for the selected trait were not. Furthermore, the apparent selection differentials on body weight appear to have been caused by size-selective fishing. For example, annual mean body weights of pink salmon removed by size-selective fishing gear (e.g., gill nets) were often considerably larger (e.g., >10%) than those of fish removed by nonselective gear (i.e., seine gear) and the mechanism for the capture of individuals of larger than average size by gill nets was understood (Todd and Larkin 1971; Ricker et al. 1978; McAllister et al. 1992).

McAllister et al. (1992) used an extension of eq. 1 to regress observations of responses of body weight on observations of apparent selection differentials in body weight to estimate "heritability of growth rate" (equivalent to $h_{y,x}^2$ in eq. 7). Estimates of the quantity, $h_{y,x}^2$, were then used to assess whether historic declines in body weight could have been caused by selective fishing and to predict potential responses in body weight to selective fishing in the future. Because of uncertainties in the analysis of historical data, all evaluations treated genetic selection by fishing gear as a hypothesis rather than a certainty. A key assumption of the equations used, however, is that body weight is highly correlated genotypically and phenotypically with traits selected by size-selective fishing gear (i.e., the values for r_A and r_P are high). While this appears reasonable, it has not been empirically evaluated.

Providing that the selected trait(s) were positively identified, estimates of r_P could be obtained from pink salmon in natural populations. Estimates of r_A could perhaps be most easily obtained by applying experimental methods reviewed in Falconer (1989, p. 315) to pink salmon in hatchery situations. However, Cheverud (1988) suggests that on theoretical grounds the values for r_A and r_P could be expected to be very similar. His review indicates that estimated values for r_A are often very similar to, if not higher than, values for r_P (particularly in studies with the most rigorous sampling protocols). Therefore, in the absence of any experimental work, the value for r_A for pink salmon could be assumed to be as high as or higher than the estimate of r_P, once the latter was obtained.

In the proposed size-selective fishing experiments (McAllister et al. 1992), statistical power (i.e., the probability of correctly detecting genetic causation in changes in body size, if it existed) would be lower using a correlated trait rather than a directly selected trait and would depend on how closely values for r_A and r_P approached 1. However, the statistical power estimates in McAllister et al. (1992) are unlikely to be positively biased. This is because the values for variance and $h_{y,x}^2$ that were used to compute statistical power were estimated by fitting the correlated model (effectively eq. 7) to the historical data (assuming that the quantity estimated in future experiments is $h_{y,x}^2$ not h_y^2). In contrast, statistical power in fishing experiments, if they were to be implemented, could be increased if it were possible to identify and precisely measure the traits directly selected by fishing.

Finally, the works of Ricker et al. (1978), McAllister et al. (1992), and McAllister and Peterman (1992) have attempted to quantify the potential impacts of selective fishing on traits (e.g., body weight) that have biological and economic significance. Quantification of the impacts of size-selective fishing on body weight can be particularly useful because estimates of changes in body weight in a population can be used to quantify potential impacts of selective fishing on population dynamics and the economic yield from the fishery (McAllister and Peterman 1992). Such analyses can be useful for helping to guide choices over alternative fishing policies that deal with the issue of size-selective fishing.

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Commentary on McAllister and Gillis (1996) concerning "Estimation of selection differentials from fish scales: a step towards evaluating genetic alteration of fish size in exploited populations"[1]

A. Kathuria, G.N.G. Gordon, and A.K. Sheridan

We wish to discuss the differences between the approaches of Sheridan (1996) and McAllister and Gillis (1996) in deriving formulae for predicting the response to selection using correlated selection differential. McAllister and Gillis replace S_x of their eq. 2b, namely $R_x = h_x^2 S_x$, with the expected value of S_x given the observed value for S'_y, which they obtain from the regression of S_x on S_y. Sheridan (1996), on the other hand, uses the regression of S_y on S_x, but inverts this relationship when making the substitution. The resolution of the contradiction between the two approaches thus depends on which is the appropriate regression to use. The advice given in the statistical literature is that the appropriate regression to use depends on which quantities are assumed known and which are in error. Thus, for instance, Williams (1959, p. 90) states, "One point on which there is sometimes uncertainty is the regression equation to be employed, that is, which variable is to be treated as the dependent variable. It must first be emphasized that the dependent variable has to be subject to random error in order that the theory on which the method of estimation of the regression equation rests may be applicable; a variable which is errorless . . . cannot be chosen as dependent variable."

Which variable then should be assumed to be in error? The direction of the regression used by McAllister and Gillis (1996) implicitly assumes that the appropriate error is the error made when inferring the selected trait from the measured correlated trait, i.e., that the error term relates to a measurement or prediction process. The method of Sheridan (1996), on the other hand, considers the underlying biological process as the one for which the regression and the associated error term is relevant; i.e., the independent variable is the selection differential of the selected trait, while the dependent variable with the associated error is the apparent selection differential of the correlated trait. As the selection pressure is applied on the actual (though unknown) selection trait, it seems more appropriate to regard it as the independent variable, taken to be without error in the regression. (The very term "correlated trait" in fact also argues for the correlated trait as being considered to be the dependent term, with error). Suppose our model is

$$Y = \alpha + \beta X + \varepsilon$$

and for a specified value of Y, y_0 say, we wish to obtain a predicted value of X. This is known as the calibration problem. There are two main approaches for obtaining the predicted values for this model. One is classical regression and the other linear inverse regression. In the classical regression approach

$$\hat{X}_c = (y_0 - \hat{\alpha})/\hat{\beta}$$

where $\hat{\beta} = s_{xy}/s_{xx}$ and $\hat{\alpha} = \bar{y} - \hat{\beta}\bar{x}$,

$$s_{xy} = \sum_1^n (x_i - \bar{x})(y_i - \bar{y}),\ s_{xx} = \sum_1^n (x_i - \bar{x})^2.$$

The linear inverse regression approach (sometimes called the "wrong regression") uses the line relating X to Y where the coefficients are obtained by formal least squares, in contrast to the classical regression of Y on X. Thus, the inverse regression equation

$$X = a + bY$$

has $b = s_{xy}/s_{yy}$ and $a = \bar{x} - b\bar{y}$, where s_{xy} is the same as above and

$$s_{yy} = \sum_1^n (y_i - \bar{y})^2.$$

From the above we obtain the inverse regression estimate,

$$\hat{X}_I = a + by_0.$$

From this point of view, the regression method used by Sheridan (1996) is the classical one and that of McAllister and Gillis (1996) is the linear inverse regression. There has been consid-

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A. Kathuria,[2] G.N.G. Gordon, and A.K. Sheridan. NSW Fisheries Research Institute, Cronulla, NSW 2230, Australia.

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[2] Author to whom all correspondence should be addressed. e-mail: gordong@fisheries.nsw.gov.au

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erable debate in the statistical literature regarding the possibility, in the calibration context, of using linear inverse regression (Brown 1986). Despite some simulation results quoted in Krutchkoff (1967) it appears (Williams 1969; Halperin 1970; Shukla 1972), that the classical calibration estimators are preferable.

Brown (1986) noted that the controversy between the use of the classical and inverse calibration estimators has given way to placing more attention on a closer specification of the problem. Perhaps this is what needs to be done here.

The statement of McAllister and Gillis (1996) that "smallest phenotypic correlations . . . between the selected and correlated traits will result in the strongest relationship between the response of the selected trait and the selection differential in the correlated trait" is not convincing as S'_y is not independent of r_p. It is the ratio S'_y/r_p that should be considered, as S'_y will become small when r_p is small.

Furthermore, if Sheridan's approach is used, then

$$(1) \quad R_x = \frac{h_x^2}{r_p}\frac{\sigma_{p(x)}}{\sigma_{p(y)}} S'_y$$

(Sheridan 1996) where h_x^2 is the heritability of the selected trait, r_p is the phenotypic correlation between the selected and correlated traits, and $\sigma_{p(x)}$ and $\sigma_{p(y)}$ are the phenotypic standard deviations in the selected and correlated traits, respectively. The response of the correlated trait is

$$R'_y = b_{(A)_{yx}} R_x$$

(Falconer 1989, p. 318). Substituting $b_{(A)_{yx}} = r_A\sigma_{A(y)}/\sigma_{A(x)}$ and R_x from eq. 1 leads to

$$R'_y = \frac{r_A\sigma_{A(y)}\sigma_{P(x)}\, h_x^2}{r_P\sigma_{A(x)}\sigma_{P(y)}} S'_y$$

where r_A is the genetic correlation coefficient between the two traits, and $\sigma_{A(x)}$ and $\sigma_{A(y)}$ are the genotypic standard deviations of the selected and correlated traits, respectively.

Since $h_x^2 = \sigma_{A(x)}^2/\sigma_{P(x)}^2$, substituting this in the above equation gives

$$R'_y = \frac{r_A\sigma_{A(y)}\sigma_{P(x)}\sigma_{A(x)}^2}{r_P\sigma_{A(x)}\sigma_{P(y)}\sigma_{P(x)}^2} S'_y,$$

which on simplification gives

$$(2) \quad R'_y = \frac{r_A h_x h_y}{r_P} S'_y$$

where $h_x = \sigma_{A(x)}/\sigma_{P(x)}$ and $h_y = \sigma_{A(y)}/\sigma_{P(y)}$ are the square roots of the heritabilities of the selected and correlated traits, respectively.

This is the same as a minor rearrangement of eq. 19.8b of Falconer (1989, p. 320), but eq. 6 of McAllister and Gillis (1996), namely

$$R'_y = (r_A r_P h_x h_y)\, S'_y$$

is different from that of Falconer. Falconer presents his eq. 19.8b as the ratio of the correlated response to the correlated selection differential, namely

$$R'_y / S'_y = (r_A h_x h_y) / r_P.$$

From a genetical point of view, Falconer's equation is intuitively correct, since the correlated response is directly proportional to the genetic correlation and the correlated selection differential is directly proportional to the phenotypic correlation. This supports Sheridan's approach. Incidently, the "useful identities" McAllister and Gillis obtain from their eq. 6 by setting $r_A = r_P = 1$ can be similarly obtained from eq. 2.

McAllister and Gillis also refer to Cheverud (1988) who conjectured that in some situations, the phenotypic correlation can be used as an estimate of the corresponding genetic correlation. We recommend that anyone contemplating this substitution of phenotypic for genetic correlations consult Willis et al. (1991) and Roff (1995). It would make a geneticist's life a lot easier when designing selection programs if the phenotypic correlation invariably closely approximated the genetic correlation between pairs of traits. Sadly, this just is not so. However, we strongly support McAllister and Gillis's statement that "quantitative approaches to assessing genetic and other changes of selective fishing are both desirable and appropriate." For another approach to the monitoring of genetic change in wild fish populations, see Sheridan (1995).

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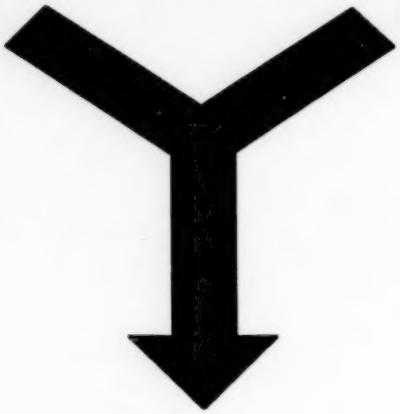

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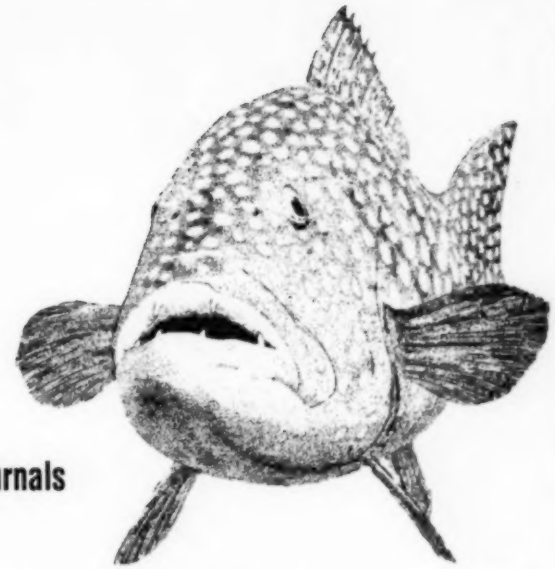

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